Doctoral Programme in Clinical Research
Pediatric Graduate School and Pediatric Research Center
Children's Hospital

University of Helsinki
Finland

CONGENITAL CYTOMEGALOVIRUS INFECTION IN FINLAND

Laura Puhakka

ACADEMIC DISSERTATION

To be presented, with the permission of the Faculty of Medicine of the University of Helsinki, for public examination in Lecture Hall 2, Biomedicum, on 14th of June 2019, at 12 noon.

Helsinki 2019

Supervisor

Professor Harri Saxen
Department of Pediatric Infectious Diseases
New Children's Hospital, Helsinki University Hospital
University of Helsinki
Helsinki, Finland

Reviewers

Docent Veijo Hukkanen
Department of Virology
University of Turku
Turku, Finland

Docent Kaarin Mäkikallio
Department of Obstetrics and Gynaecology
Turku University Hospital
University of Turku
Turku, Finland

Opponent

Professor Liisa Lehtonen
Department of Pediatrics
Turku University Hospital
University of Turku
Turku, Finland

The Faculty of Medicine uses the Urkund system (plagiarism recognition) to examine all doctoral dissertations.

ISBN 978-951-51-5231-2 (paperback)
ISBN 978-951-51-5232-9 (PDF)
http://ethesis.helsinki.fi

Unigrafia
Helsinki 2019

To all children with congenital CMV infection

ABSTRACT

Congenital cytomegalovirus infection (cCMV) is the most common congenital infection of the fetus. It affects approximately 6 in 1,000 of all newborns in developed countries. The prevalence varies in different populations. Only a minority of the infected infants, approximately 10%, have symptoms due to cCMV at birth. The morbidity among these symptomatic cCMV infants is high; about half of them will develop permanent long-term sequelae, such as hearing loss or neurological impairment. The majority of cCMV-infected infants are asymptomatic, and their prognosis is clearly better. It is estimated, however, that 10%–15% of the asymptomatic infants also develop some long-term sequelae due to the infection. Congenital CMV infection is the most common non-genetic cause of sensorineural hearing loss (SNHL) in young children. In addition, it is estimated to be the most common infectious cause of intellectual disability among children.

The seroprevalence of CMV in the population is an important indicator of the frequency of cCMV. The fetus can be infected in the womb both after maternal primary and non-primary CMV infection. Primary infection occurs when a person encounters the CMV for the first time. Non-primary infection means either a reactivation of a latent virus or a re-infection with a different strain of the virus in a seropositive person. If the infection is common in the population, both reactivations and primary infections are subsequently frequent.

We have studied the disease burden of cCMV in Finland. For that purpose, we evaluated the seroprevalence for CMV in Finland and the outcome of infants with symptomatic and asymptomatic cCMV infection. We also analyzed whether the maternal CMV infection during pregnancy causing cCMV was primary or non-primary.

In the first study, we evaluated CMV seroprevalence and the temporal changes in the seroprevalence in Finnish pregnant women. We examined CMV serum antibodies of 200 randomly collected samples from the Finnish Maternity Cohort (FMC) serum bank during three different decades: 1992, 2002, and 2012. The seropositivity rate decreased significantly from 84.5% (95% CI 78.7–89.2) in 1992 to 71.5% (95% CI 64.7–77.6) in 2012.

The outcome of symptomatic cCMV infection was evaluated retrospectively from a cohort of children diagnosed with cCMV in five Finnish tertiary hospitals from 2000 to 2012. The type of the maternal infection was defined either as a primary or as a non-primary, based on the archived early pregnancy serum samples. We identified 29 infants with symptomatic cCMV from the patient registers. The FMC serum bank sample was available for 26 of them,

and the study population comprised these 26 infants. The maternal CMV infection during pregnancy had been a primary infection in less than half of the cases (12/26, 46%). Any long-term sequelae occurred in 58% (15/26) of infants, neurologic abnormality in 50% (12/24), and SNHL in 42% (8/19) of the children. Of the children whose mothers had suffered from primary infections in the first trimester, 86% (6/7) developed one or more long-term sequelae. Of the children whose mothers had experienced non-primary infections during the pregnancy, 64% (9/14) developed long-term sequelae. None of the 5 children whose mothers had had primary infections in the second or third trimester had developed any long-term sequelae.

To evaluate the prevalence of cCMV in the Finnish population and the outcome of asymptomatic cCMV infection, we performed a large-scale screening study in four Helsinki area hospitals from September 2012 to January 2015. Of the 19,868 infants screened with a saliva CMV PCR test, 40 had a confirmed cCMV infection, corresponding to a prevalence of 2 in 1,000 (95% CI 1.4–2.6). Maternal CMV infection during pregnancy had been a primary one in 47% (18/38). We followed the cCMV positive children and healthy control children for 18 months. The Griffiths Mental Development Scales were used to assess neurological outcome. No differences in the Griffiths scales could be found between cCMV positive and healthy controls at age 18 months. Hearing was evaluated by transient-evoked otoacoustic emission (TEOAE) and sound field audiometry (SF). Similarly, the hearing outcome of the cases did not differ from that of the healthy controls. None of the children had a bilateral hearing loss requiring hearing rehabilitation. In addition, no CMV-related findings were detected in the ophthalmologic examinations.

We evaluated the viral shedding of the cCMV-positive children identified in screening at 3 and 18 months of age. Urine CMV culture was positive in all samples tested at 3 months (40/40) and at 18 months (33/33). Saliva CMV PCR was positive in all 3-month samples (40/40) but in only 24% (9/37) of 18-month samples. We determined the CMV glycoprotein B (gB), gH, and gN genotypes from the CMV-positive screening samples. All previously described genotypes except gN2 could be found in our cohort of CMV positive samples. Mixed infections were uncommon (3/38).

In conclusion, our findings indicate that the disease burden of cCMV is relatively low in Finland. The prevalence was only 2 in 1,000, and the outcome of the asymptomatic infants was favourable. Although the infection was in general rare, the morbidity of the symptomatic infection was remarkable. Over half of the infants from the retrospective cohort with CMV-related symptoms at birth developed later long-term sequelae. The CMV genotype distribution of our CMV-positive population without symptoms at birth was similar to that reported from countries with a higher frequency of CMV infections and does not therefore explain the low burden of the disease in Finland.

TIIVISTELMÄ

Synnynnäinen sytomegalovirusinfektio (CMV) on yleisin sikiöaikainen infektio. Sen esiintyvyys eri väestöissä vaihtelee ja on kehittyneissä maissa noin 6/1000 vastasyntynyttä. Vain noin 10% sikiöaikana CMV-infektion saaneista vastasyntyneistä on syntyessään oireisia. Nämä lapset ovat usein sairaita, ja heistä noin puolelle jää infektiosta johtuva pitkäikaishaitta, kuten kuulovaurio tai neurologinen vamma. Suurin osa sikiöaikana infektoituneista lapsista on kuitenkin täysin oireettomia ja heidän ennusteensa on selvästi parempi. Arviolta noin 10-15%:lle näistä oireettomista lapsistakin ilmaantuu seurannassa infektion aiheuttamia pitkäaikaispulmia. Synnynnäinen CMV-infektio on yleisin ei-geneettisen kuulovaurion aiheuttaja. Lisäksi on arvioitu, että se on yleisin kehitysvammaisuutta aiheuttava infektio.

Sytomegaloviruksen esiintyvyys väestössä vaikuttaa synnynnäisen CMV-infektion yleisyyteen. Sikiö voi infektoitua kohdussa, mikäli äiti sairastaa raskausaikana ensi-infektion eli kohtaa CMV:n ensimmäisen kerran. Äidin aiemmin sairastama, elimistössä latenttina säilynyt virus voi aktivoitua raskausaikana tai aiemmin CMV-infektion sairastanut äiti voi raskausaikana saada uuden, toisen viruskannan aiheuttaman tartunnan. Myös näihin uusintainfektioihin liittyy sikiön infektoitumisen riski. Infektion ollessa yleinen väestössä, ensi-infektioita ja viruksen reaktivaatioita tapahtuu usein.

Tutkimme synnynnäisen CMV-infektion aiheuttamaa tautitaakkaa Suomessa. Selvitimme CMV:n esiintyvyyttä väestössä sekä syntyessään oireisten ja oireettomien synnynnäistä CMV-infektiota sairastavien lasten ennustetta. Selvitimme, oliko sikiöaikaiseen infektioon johtanut äidin raskauden aikainen CMV-infektio ensi-infektio vai ei.

Ensimmäisessä osatyössä selvitimme raskaana olevien naisten CMV-seroprevalenssia ja sen muutoksia. Tutkimme CMV-vasta-aineet 200:sta satunnaisesti valitusta seerumipankkinäytteestä vuosilta 1992, 2002 ja 2012. Seroprevalenssi laski vuodesta 1992, 84,5% (95%CI 78,7-89,2) vuoteen 2012, 71,5% (95%CI 64,7-77,6). Muutos oli tilastollisesti merkitsevä.

Oireisen CMV-infektion taudinkuvaa selvitimme retrospektiivisesti. Haimme Suomen yliopistosairaaloiden potilasrekistereistä ne lapset, joilla oli vuosina 2000–2012 diagnosoitu oireinen synnynnäinen CMV-infektio. Selvitimme äidin raskauden aikaisen CMV-infektion luonteen alkuraskauden seeruminäytteistä. Yhteensä 29 synnynnäistä CMV-infektiota sairastavaa lasta oli syntynyt ko ajanjaksolla. Tutkimukseen otettiin mukaan ne 26 lasta, joiden kohdalla alkuraskauden seerumipankkinäyte oli käytettävissä äidin CMV-infektion ajankohdan selvittämiseksi. Äiti oli sairastanut CMV-ensi-infektion

vajaassa puolessa tapauksista (46%, 12/26). Seurannassa oireisista lapsista 58% (15/26) kärsi jostain pitkäaikaispulmasta, 50%:lla (12/24) oli neurologinen poikkeavuus ja 42%:lla (8/19) oli kuulovaurio. Niistä lapsista, joiden äiti oli sairastanut CMV-ensi-infektion ensimmäisen raskauskolmanneksen aikana, 86%:lla (6/7) esiintyi joku pitkäaikaisongelma. Lapsista, joiden äidillä oli raskauden aikana ollut latentin infektion reaktivaatio tai uuden kannan aiheuttama uusi infektio, 64%:lla (9/14) esiintyi seurannassa poikkeavuus. Kenelläkään niistä viidestä lapsesta, joiden äiti oli sairastanut CMV ensi-infektion alkuraskauden jälkeen toisessa tai kolmannessa raskauskolmanneksessa, ei todettu seurannassa poikkeavuutta.

Laajassa seulontatutkimuksessa selvitimme synnynnäisen CMV-infektion esiintyvyyttä väestössä ja oireettoman infektion taudinkuvaa. Seuloimme vastasyntyneitä neljässä Helsingin alueen synnytyssairaalassa syyskuusta 2012 tammikuuhun 2015 syljestä otettavalla CMV-nukleiinihapon osoitustestillä. Yhteensä 19 868 lasta osallistui seulontaan ja näistä 40:lla todettiin varmennettu CMV-infektio, joten esiintyvyys väestössämme oli 2/1000 (95%CI 1.4-2.6/1000). Äiti oli sairastanut CMV-ensi-infektion 47%:ssa (18/38) tapauksista. Seurasimme tutkittavia 18 kuukauden ikään asti. CMV-positiivisten lasten ja terveiden verrokkien välillä ei todettu eroa suoriutumisessa Griffithsin kehitysseurantamenetelmän testeissä. Myöskään kuulontutkimuslöydöksissä (otoakustinen emissio, äänikenttä-audiometria) ei todettu eroa CMV-positiivisten ja terveiden verrokkien välillä. Kenelläkään ei todettu kuulonkuntoutusta vaativaa molemminpuoleista kuulovikaa. Silmälääkärin tutkimuksessa ei todettu CMV-infektioon liittyviä löydöksiä.

Selvitimme seulonnassa diagnosoitujen CMV-positiivisten lasten viruseritystä 3 ja 18 kuukauden iässä. Virtsan CMV-viljely oli positiivinen kaikissa tutkituissa näytteissä 3kk (40/40) ja 18kk (33/33) iässä. Syljen CMV-testi oli positiivinen kaikissa 3 kk näytteissä (40/40) mutta vain 24%:ssa (9/37) 18 kk näytteitä. Selvitimme CMV:n glykoproteiinien B (gB), gH, ja gN genotyyppejä CMV-positiivisista seulontanäytteistä. Lukunottamatta gN2 genotyyppiä, kaikkia muita aiemmin kuvattuja genotyyppejä löytyi aineistossamme. Sekainfektiot olivat harvinaisia (3/38).

Tutkimustemme mukaan synnynnäisen CMV-infektion aiheuttama tautitaakka oli Suomessa suhteellisen pieni. Esiintyvyys oli ainoastaan 2/1000 ja oireettomien lasten ennuste oli aineistossamme suotuisa. Vaikka infektio oli kokonaisuudessaan harvinainen, oireisen infektion aiheuttama sairastavuus oli huomattavaa. Retrospektiivisessa aineistossamme yli puolella syntyessään oireisista lapsista oli joku pitkäaikaispoikkeavuus. CMV:n genotyyppien jakauma seulonta-aineistossamme oli samankaltainen kuin väestöissä, joissa synnynnäistä infektiota on kuvattu esiintyvän enemmän. Täten genotyyppien jakauma ei selitä synnynnäisen CMV-infektion vähäistä tautitaakkaa väestössämme.

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications:

I Puhakka L, Sarvikivi E, Lappalainen M, Surcel HM, Saxen H. Decrease in seroprevalence for herpesviruses among pregnant women in Finland: Cross-sectional study of three time points 1992, 2002 and 2012. Infect Dis (Lond) 2016;48(5): 406–410.

II Puhakka L, Renko M, Helminen M, Peltola V, Heiskanen-Kosma T, Lappalainen M, Surcel HM, Lönnqvist T, Saxen H. Primary versus non-primary maternal cytomegalovirus infection as a cause of symptomatic congenital infection – register-based study from Finland. Infect Dis (Lond). 2017;49(6):445–453.

III Puhakka L, Lappalainen M, Lönnqvist T, Niemensivu R, Lindahl P, Nieminen T, Seuri R, Nupponen I, Pati S, Boppana S, Saxen H. The Burden of Congenital Cytomegalovirus Infection: A Prospective Cohort Study of 20 000 Infants in Finland. J Pediatric Infect Dis Soc. 2018 Mar 15. Epub ahead of print.

IV Puhakka L, Pati S, Lappalainen M, Lönnqvist T, Niemensivu R, Lindahl P, Nieminen T, Seuri R, Nupponen I, Boppana S, Saxen H. Viral shedding, and distribution of cytomegalovirus glycoprotein H (*UL75*), glycoprotein B (*UL55*), and glycoprotein N (*UL73*) genotypes in congenital cytomegalovirus infection. Submitted.

The publications are referred to in the text by their roman numerals. The articles have been reprinted with the permission of the copyright holders. In addition, some unpublished data are presented.

ABBREVIATIONS

CI	Confidence interval
CMV	Cytomegalovirus
cCMV	Congenital cytomegalovirus infection
CMV-HIG	Cytomegalovirus hyperimmunoglobulin
CNS	Central nervous system
CT	Computerized tomography
DBS	Dried blood spots
FMC	Finnish Maternity Cohort
gH	Glycoprotein H
gN	Glycoprotein N
gB	Glycoprotein B
HSCT	Hematopoietic stem cell transplantation
MRI	Magnetic resonance imaging
PCR	Polymerase chain reaction
SCID	Severe combined immunodeficiency
SF	Sound field audiometry
SNHL	Sensorineural hearing loss
TEOAE	Transient-evoked otoacoustic emission
UAB	University of Alabama, Birmingham
US	Ultrasound

1 INTRODUCTION

Cytomegalovirus (CMV) is a ubiquitous virus, presenting all over the world. In most countries, the infection is acquired before adulthood. When acquiring CMV infection for the first time, the person experiences a primary infection. After primary infection, the host gets viremic, and the virus can be excreted to bodily fluids such as saliva, urine, genital secretions, and breast milk for variable periods of time. After primary infection, the virus remains latent in the body and can recurrently be reactivated and thus shed through excretions again.

Primary CMV infection in immunocompetent children and adults is usually asymptomatic or presents with flu-like symptoms. Most CMV seropositive persons have encountered the infection without knowing it. In immunocompromised individuals, neonates, or a developing fetus, the virus may cause clinical disease and lead to significant morbidity.

The fetus can be infected already in the womb if the mother has either primary or non-primary CMV infection during pregnancy. Non-primary infection means that the seropositive mother has either reactivation of the latent infection or encounters a new infection with a new strain of the virus.

CMV infection is the most common congenital infection in developed countries. It has been considered the most common infectious cause for intellectual disability. It is also the most common non-genetic cause of sensorineural hearing loss in small children. Only a minority of infants with congenital CMV infection (cCMV) have symptoms at birth. Symptomatic cCMV has high long-term morbidity. About half of the infants with symptomatic cCMV will develop some long-term sequelae due to the infection. The majority of infected children, however, appear healthy at birth. The prognosis of these asymptomatic infants is much better, and they usually recover without sequelae. The reasons for the great variability of cCMV-related sequelae, from no symptoms in most cases, to severe mental retardation and deafness in some cases, is not clear.

Due to the disease burden of cCMV, developing a vaccine for CMV has been advocated as a high priority. However, major obstacles in vaccine development exist, and after decades of research, there is still no vaccine on the market. In order to identify asymptomatic infants, universal screening of all newborns has been suggested to allow early interventions. To evaluate the benefits of possible future vaccinations, or universal screening of all newborns, it is essential to know the local burden of cCMV. In this thesis, we have evaluated the disease burden of cCMV in Finland.

2 REVIEW OF THE LITERATURE

2.1 CYTOMEGALOVIRUS

2.1.1 HISTORY

Doctor Hugo Ribbert was the first one to notice large inclusion bodies in the kidneys in a stillborn infant with syphilis in 1881. He reported the findings in 1904 [1]. He could, however, interpret these finding only after Jesionek and Kiolemenoglou had described similar findings in the lungs, kidneys, and liver of an 8-month old fetus [2, 3]. In 1950, Wyatt et al introduced the term "Cytomegalic inclusion disease" [4]. They described the postmortem findings in six infants with the inclusion disease. After reviewing the literature of previously described cases, the authors concluded that the etiological agent must be a specific virus infecting fetus and infants. The morphology and cytology of the inclusion-bearing cells is pathognomonic of the disease [4, 5]. The virus was later isolated in 1956/57 by several researchers [6-8]. In 1965, Klemola and Kääriäinen recognized CMV as a cause of mononucleosis-like illness [2, 9].

2.1.2 STRUCTURE

Human CMV is a double-stranded DNA virus. It belongs to the beta-herpesvirus family and is the largest herpesvirus. The schematic structure of CMV is presented in Figure 1. CMV is composed of three layers. In the middle is the nucleus with the genome packed tightly in an icosahedral protein capsid, surrounded by a proteineus tegument layer that is enclosed by a lipid envelope [10, 11]. The nucleus contains a 235-kilobase genome with over 166 genes. The genome consists of two regions: unique long (UL) and unique short (US). The genes are named by prefix from the location (UL/US) and a sequential number [12]. Tegument contains proteins such as pp65, pUL47, pUL48, pp150, pp28, and pp71. Tegument proteins have a role in stabilizing the structure, delivering the viral genome to the nucleus, viral replication, and immune evasion. The outermost lipid envelope contains several glycoproteins that associate in complexes and play a role in virus–host interaction [13, 14].

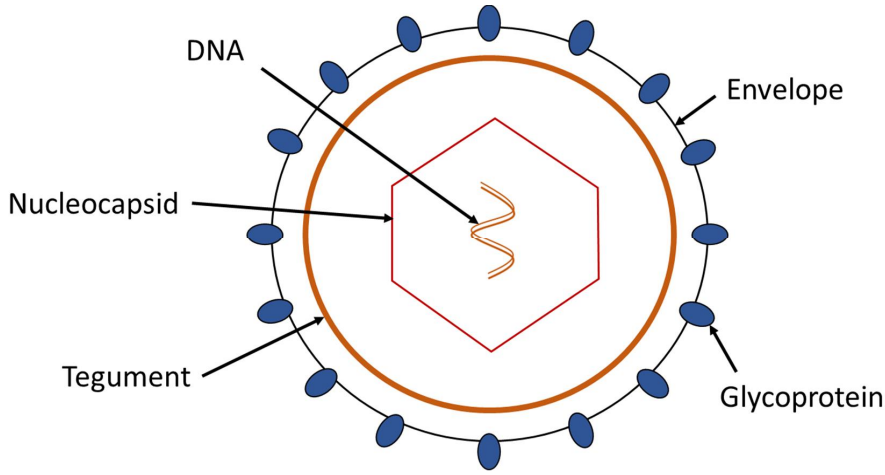

Figure 1 Schematic structure of cytomegalovirus

2.1.2.1 Strains/Genotypes

There is a wide variability in the genome of CMV. It has been hypothesized that the genetic polymorphism in the viral genes encoding for proteins involved in the host immune response and virulence factors may contribute to the variability of clinical outcome of cCMV [12]. However, the evidence supporting this hypothesis is controversial [15-27].

Variability occurs in the CMV genes encoding for envelope glycoproteins gB (*UL55*), gH (*UL75*), gN (*UL73*), and gO (*UL74*), and cytokine/chemokine homologs, tumor necrosis factor-α like receptor gene (*UL144*), α-chemokine genes *UL146* and *UL147*, and functional β-chemokine receptor *US28*. These genes have been used to define different genotypes and strains of the virus. The virus can be genotyped for one or more genes. The generally approved consensus defining clinically relevant strains is still lacking [12].

The presence of more than one genotype in one sample, reflecting mixed infection with several strains, is common among immunocompromised individuals [28-31]. Mixed infections also exist in cohorts of congenitally infected infants [15, 32-36]. However, the clinical role of mixed infections versus an infection caused by a single strain is open. Mixed infections have occurred among infants with both non-symptomatic and symptomatic cCMV [15, 32-36].

2.1.2.2 Glycoproteins gB (UL55), gH (UL75), and gN (UL73)

Envelope glycoprotein B (gB) is the major component of the lipid envelope. The gene coding for gB is *UL55*. This protein is highly conserved in all herpes

family viruses. It is thought to be essential for the life cycle of the virus. It has an important role in both the virus entry and cell-to-cell spreading of the virus [37]. Four main gB variants, gB1–gB4, have been identified.

Envelope glycoprotein H (gH) and its complexes with other surface glycoproteins are involved in the fusion of the viral and host cell membranes. This step is essential for the entry of viral material into the cell [38, 39]. The gene coding for gH is *UL75*. Two variants, gH1 and gH2, have been identified.

Envelope glycoprotein N (gN) is essential for virus replication. It is involved in virus attachment to the host after forming a highly immunogenic complex with glycoprotein gM [40-43]. The gene coding for gN is *UL73*. The glycoprotein gN is highly polymorphic and 7 gN genomic variants have been identified: gN-1, gN-2, gN-3a, gN-3b, gN-4a, gN-4b, and gN-4c [40, 44, 45].

Both gB and the pentamer complex containing gH are also highly immunogenic and have been used in vaccine development as potential antigens [46].

2.2 EPIDEMIOLOGY

CMV exists all over the world and causes congenital and acquired infections. The seroprevalence for CMV among women of reproductive age in different populations is presented in Table 1. It has been lowest in western European countries such as France (46%), Ireland (37%), and the Netherlands (37%) [47-49]. In many areas, especially in developing countries, the prevalence has been very high and approaches 100% [50-52]. In Finland, the seroprevalence among pregnant women has been 56.3%–76.4% [53, 54]. Seropositivity has been associated with socioeconomic factors; that is, it is higher among people with lower socioeconomic status [53, 55-57].

Table 1. *CMV seroprevalence among women of child-bearing age in different populations.*

Location	CMV seroprevalence	Population studied	Year / ref
Norway	54%	Pregnant women	2018/[58]
Sweden	72%	Pregnant women	2008/[59]
United Kingdom (UK)	77.1% All 49% White British women 89% South Asian origin, born in UK 98% Born in Asia	Pregnant women	2013/[60]
Ireland	37%	Women age 20-39 y	2016/[48]
France	45.6% All 27.6%-48.6% Born in Western country 96.6%-99.5% Born in non-Western country	Women age 15-49 y	2017/[47]
Germany	51.7%	Women age 18-45 y	2018/[61]
Italy	79.9%	Pregnant women	2006/[62]
Portugal	75.5%-81.5%	Women age 20-44 y	2011/[63]
The Netherlands	36.9% Dutch / Western origin 85.1% Non-Western migrants	Women age 20-45 y	2015/[49]
Austria	73.2% All 53% Born in Western Europe 92% Born in Eastern Europe 96% Born in Middle East	Pregnant women	2015/[64]
United States (USA)	61.3% All 55.8% Born in USA 90.2% Born outside of USA	Women aged 20-49 y	2016/[65]
China	96.2%	Infant DBS[a]	2017/[51]
Japan	69.1%	Pregnant women	2015/[66]
Iran	92%	Blood donors and healthy subjects[b]	2015/[67]
Yemen	91.3%	Pregnant women	2016/[68]
Mexico	89.6%	Pregnant women	2018/[69]
Brazil	98.1%	Pregnant women	2018/[52]

DBS=Dried blood spot, y=years

[a]IgG measured in infant's blood sample reflect maternal IgG transferred through placenta during the third trimester

[b]Meta-analysis

2.2.1 PREVALENCE OF CONGENITAL CMV

CMV is the most common cause of congenital infections in developed countries. In universal screening studies from mainly industrialized countries, the overall prevalence of cCMV has been 0.64%–0.7% [70, 71]. The prevalence of cCMV infection is known to correlate with the seroprevalence of CMV in the population. The higher the seroprevalence, the higher the prevalence of cCMV [70, 72, 73]. This is in contrast to the epidemiology of two other pathogens causing congenital infections: Rubella and Zika viruses. It has been shown that incidence of congenital Rubella and Zika infections dramatically drops after the seroprevalence in a community has reached a certain level. This is explained by the protective role of specific antibodies [74, 75]. However, two features in CMV are different from Zika and Rubella. First, CMV immunity does not give full protection against re-infections with different strains. Second, after the primary infection, CMV remains in the host as a life-long latent infection leading to recurrent reactivations and shedding of infectious virus.

The prevalence of cCMV in different populations is presented in Table 2. Maternal seroprevalence in the community affects the prevalence of cCMV, meaning that also prevalence for cCMV is higher in communities with a low standard of living, crowded accommodation, and lower hygienic standards [70]. In older studies, the screening was performed with CMV culture-based regimens, which are time consuming but serve as the gold standard in assessing cCMV infection. Polymerase chain reaction (PCR)-based methods have replaced the culture in most cohorts. PCR, however, is sensitive to contamination and the proportion of false positives has been high in studies with confirmatory sampling. In the study from Portugal reporting high cCMV prevalence of 10.1 in 1,000, the screening was performed from the archived DBS cards [76]. Due to the study design, resampling could not be performed, but the original samples were studied in triplicate and the positive samples were retested for confirmation [76]. In other studies reporting high prevalence of 10 and 12 in 1,000 in Brazil, all PCR-positive samples were either retested [77] or confirmed by CMV culture [78]. In addition, PCR-based screening studies in a CMV-seropositive population identified a very high cCMV prevalence in Gambia (54 in 1,000) and in China (61 in 1,000) [79, 80]. Both presented populations with a very low standard of living. Coexisting infections may influence the prevalence, as active placental malaria infection correlated with cCMV infection in Gambian cohort. However, in these two studies from Gambia and China, control sampling was not performed, and thus false positives may have influenced the findings. The lack of infrastructure may also affect the quality of diagnostics in developing countries.

Table 2. *Prevalence of cCMV and proportion of symptomatic cCMV in different countries.*

Country (ref)	Screening method	Individuals screened, n	Prevalence, n/1,000	Proportion of symptomatic (%)
UK [81]	Throat swab culture	4,178	1.7	NA
UK [82]	Throat swab culture	14,200	3	12% (5/42)
Ireland [83]	Urine or saliva, PCR	1,044	1.9	0/2
Italy [84]	Saliva culture	1,268	4.7	0/6
Italy [62]	DBS, PCR	9,032	1.8	13% (2/16)
France [85]	Saliva, PCR	11,715	3.7	20% (9/44)
Portugal [76]	DBS, PCR	3,600	10.1	NA
Belgium [86]	Urine culture	14,021	5.3	5% (4/74)
The Netherlands [87]	DBS, PCR	6,433	5.4	NA
Sweden [88]	Urine culture	16,474	4.6	22% (17/76)
Slovenia [89]	Urine PCR	2,841	1.4	0/4
Israel [90]	Saliva	9,845	4.9	22% (10/46)
USA [91]	Saliva rapid culture and/or PCR	100,332	3.9	9% (28/313)
Japan [92]	Urine culture	11,938	3.1	14% (5/37)
Japan [93]	Urine on filter paper, PCR	21,272	3.1	30% (20/66)
Japan [94]	DBS, PCR	1,176	1.7	0/2
Japan [95]	Urine, PCR	23,368	2.6	3% (2/60)
Japan [96]	Urine on filter paper, PCR	6,348	5	50% (16/32)
Japan [97]	Urine on filter paper, PCR	2,193	4.6	40% (4/10)
Brazil [78]	Saliva or urine, PCR	12,295	10	10% (12/121)
Brazil [98]	Saliva, urine PCR	1,200	12	4% (1/25)
China [51]	Saliva or DBS, PCR	10,933	6.9	3% (2/75)
China [79]	Urine PCR	1,159	61	24% (17/71)
Iran [99]	Urine PCR	1,617	4.9	38% (3/8)
India [100]	Saliva PCR	750	4	33% (1/3)
Gambia [80]	Urine PCR	741	54	NA

NA=not available

2.2.2 TRANSMISSION

2.2.2.1 Horizontal transmission

After primary CMV infection, infectious virus is shed to blood, saliva, urine, breast milk, sperm, and the cervix. The primary infection leads to life-long latency, and the infection can periodically reactivate to cause recurrent viral shedding [14, 101]. Transmission occurs horizontally from person to person or vertically from mother to the fetus.

Young children are regarded as the main reservoir of infectious virus in the community. Young children acquiring the infection in early years or already during their fetal life continue to excrete the virus for long periods of time, up to several years. Excretion is common during the second year of life and gradually becomes less common during later years in childhood [81, 101-105]. However, recurrent shedding has also been reported in the adult population [106, 107]. Children attending daycare centers were more often shedding (13%–83%) compared to those who were taken care of at home [102-105]. Especially for pregnant women, the main source of the infection is infected toddlers [14, 101].

2.2.2.2 Vertical transmission

If the mother has contracted the virus during pregnancy and develops a primary CMV infection, the overall risk of a fetal infection is about 40%. Transmission rates vary, however, according to the trimester of pregnancy: 37% in the first trimester, 40% in the second trimester, and 65% in the third trimester [108, 109]. On the other hand, if the mother is already CMV-positive before pregnancy, the fetus can be infected due to reactivation of maternal latent infection. In addition, the seropositive mother can be re-infected by a new strain of CMV. In the case of a non-primary infection, the risk of fetal infection has been estimated to be 0.5%–3.4% [70, 110, 111]. In non-primary infections, the risk of infection of the fetus is thus much lower compared to the primary infection. Non-primary infections are, however, relatively common among pregnant women, and a significant proportion of congenital infections are due to maternal non-primary infections [73, 85, 97, 112, 113].

2.2.2.3 Virus transmission and circulation in the community

CMV circulates ubiquitously in the community, since the CMV positive individuals continue to shed the virus for a long time and recurrent shedding occurs. The circulation of the virus in the community is presented in Figure 2.

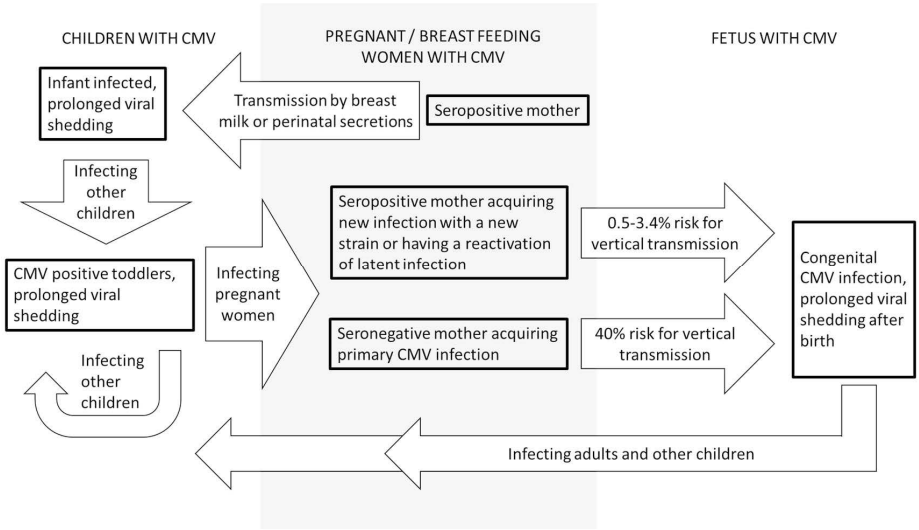

Figure 2 Simplified figure on routes of CMV transmission in the community. Young children are the main reservoir of infectious virus leading to horizontal transmission. Transmission occurs after exposure to secretions containing viruses, such as breast milk (breastfed infants), saliva and urine (all age groups), and genital secretions (perinatal infections, sexually active teenagers, and adults). Transmission through blood transfusion and solid organ transplantation is also possible.

2.3 PATHOGENESIS

Due to its broad tropism, CMV may infect almost all cell types in the body, causing a wide spectrum of end organ diseases. The virus can replicate at least in epithelial, endothelial, macrophage, and dendritic cells as well as in parenchymal and connective tissue cells of almost all organs. However, the immune system of immunocompetent hosts can limit the infection in the early phase, and the primary infection is usually asymptomatic. Primary CMV infection induces both innate and adaptive immunity [114-116].

The virus enters the body through body surfaces after direct contact with virus containing secretions. The various membrane proteins, membrane glycoproteins, and the established glycoprotein complexes are essential for HCMV entry into the cells. After binding to cell surface receptors, the virion envelope fuses with the cellular membrane and the nucleocapsid is released into the cell. In the cytoplasm, the nucleocapsid is actively transported toward the nucleus. Viral gene expression starts after the viral genome has reached the nucleus. New nucleocapsids with viral DNA are produced in the nucleus and are then encapsulated in two steps starting in the nucleus and completed

in the cytoplasm. Mature virions are transported to the cell surface and released from the cell [13, 14, 114, 117].

In spite of the robust immune response after primary infection, CMV is able to establish a life-long latency. The mechanism of latency is not completely understood. During the latent infection, the viral genome is in episomal form in the human cell and lytic infection is inhibited. Suppression of the major immediate early promoter is essential for keeping the virus in latent phase. CMV latency occurs at least in myeloid lineage cells, myeloid dendritic progenitors, and peripheral monocytes. Latency may also occur in endothelial and neuronal progenitors [118, 119]. Recurrent reactivations with production of infectious virus occur only in differentiated macrophages and dendritic cells [114, 119]. The reactivation is normally suppressed by the immunoresponse of the immunocompetent host. Long-term memory T cells have an important role in preventing clinical disease after recurrent reactivation of the virus [116]. However, in the case of immunosuppressed individuals or pregnant women with a developing fetus, uncontrolled viral replication can take place and lead to major morbidity.

Several pathological mechanisms are involved in fetal damage caused by intrauterine CMV infection. Both direct cytotoxic effect of the virus, as well as immunoresponse of the host are involved in the cellular damage. CMV infection may modify apoptotic and cell cycle pathways that are essential to normal embryogenesis. Endovascular injury, as well as placental infection leading to placental insufficiency, may lead to hypoxia in the developing organs [120, 121].

2.4 ACQUIRED CMV INFECTION

2.4.1 IMMUNOCOMPETENT INDIVIDUALS

CMV infection in immunocompetent individuals is usually a benign self-limiting condition with mild, if any, symptoms. Most primary infections in adults, and especially among pregnant women, are asymptomatic. The symptoms that do occur can be unspecific such as fatigue, malaise, fever, myalgias, headache, night sweats, and hepatitis. CMV can cause a mononucleosis-like illness with a milder pharyngitis and lymphadenopathy compared with mononucleosis caused by Epstein-Barr virus [122-125]. In immunocompetent children, the disease is usually mild and most infections are asymptomatic. Some severe CMV-induced pneumonias have been reported also in immunocompetent children [126, 127]. In most cases, however, CMV detection in the bronchoalveolar fluid of a previously healthy

child indicates a reactivation of a latent infection, not acute CMV infection [128]. CMV can also cause hepatitis, which is usually a self-limited condition [129, 130]. CMV infection may induce thrombocytopenia either through direct infection of megakaryocytes or an indirect immunomediated mechanism [131-133]. In adults, CMV has been associated with venous thromboembolic events such as splanchnic vein thrombosis, with a favourable outcome in most cases [134, 135]. In addition, CMV has been identified as a causative agent in colitis. Among immunocompetent persons, the interpretation of positive CMV detected in the colitic gut is not always clear. The virus can be either an innocent bystander or a sign of an undiagnosed immunodeficiency. In rare cases, it is the only cause of the colitis [136, 137].

2.4.2 IMMUNOCOMPROMISED INDIVIDUALS

The CMV infection causes significant morbidity and mortality both in solid organ transplant and hematopoietic stem cell transplant (HSCT) recipients and other patients with immunocompromised status [138, 139]. In the pediatric population, infants with severe primary immunodeficiency such as severe combined immunodeficiency (SCID) are especially vulnerable to CMV. The prevention of infection before stem cell transplantation is essential, since the outcome of HSCT has been worse in children with SCID and active infection at the time of transplantation [140, 141].

CMV infection in immunocompromised persons can present as a systemic disease or an end-organ CMV disease of almost any organ, such as pneumonia, gastrointestinal disease, hepatitis, retinitis, encephalitis, nephritis, cystitis, myocarditis, and pancreatitis. The diagnosis is based on the clinical signs and symptoms of the organ together with viral detection. Serology is not reliable in assessing the history of CMV infection in severely immunocompromised patients, as they do not always develop CMV-specific antibodies. In case of retinitis, the clinical picture itself is so typical that an experienced ophthalmologist can recognize the infection without isolation of virus [138]. Both primary and recurrent infections may cause disease in immunocompromised individuals. Clinical symptoms correlate with the viral load. Thus, low-grade viremia may be asymptomatic and high viral load usually correlates with severe symptoms. Pre-emptive antiviral therapy of asymptomatic viremic patients with immunosuppression does reduce cases of severe CMV disease [138, 142].

2.4.3 PERI- AND POSTNATAL INFECTIONS IN NEONATES

The transmission from mother to neonate can occur perinatally from genital secretions, or postnatally. The most common source of postnatal infection is

breast milk. Sensitive PCR techniques have detected CMV or CMV DNA in 67%–97% of the milk of CMV seropositive mothers [143-150]. Of the preterm infants who received untreated breast milk of seropositive mothers, 19% acquired postnatal CMV infection [151]. The proportion ranged from 6%–37% in different cohorts [145, 151-155]. Less data has been reported from term infants. Granström et al evaluated 148 mother child pairs in Finland, and postnatal CMV infection had occurred by 4 months of age in 38% of breastfed children whose mother was seropositive [156]. In an African study by Musonda et al., the transmission of CMV was associated with the length of breast feeding in a seropositive, HIV-negative population [150]. CMV infection occurred by the age of 18 months in 110/119 (92%) of the children who continued to receive breast milk at the age of 18 months, and in 25/32 (78%) of the children who were breast fed for less than one year [150]. The postnatal infection is usually asymptomatic. In premature infants, however, symptoms are more common. Any CMV-related symptoms, including blood count abnormalities, petechiae, hepatomegalia, hyperbilirubinemia, elevated transaminases, jaundice, or CMV-pneumonia, occurred in 0%–89% of premature infants with acquired CMV infection. A sepsis-like syndrome, however, was less common, occurring in 0%–25% of the infected preterm infants [145, 152-155]. Cases of colitis due to post- or perinatal infection have been described, but these cases are extremely rare [157]. In some studies, postnatal CMV infection among preterm infants has been associated with inferior neurological outcome [158, 159]. In most studies, however, the post- and perinatal infection did not affect the long-term outcome or cause hearing loss [148, 155, 160-163].

2.5 CONGENITAL CMV INFECTION

2.5.1 MANIFESTATIONS

Transmission of CMV infection can occur transplacentally from mother to developing fetus leading to cCMV. Most children with cCMV infection are totally asymptomatic at birth. Only about 10%–15% of infants with cCMV have symptoms due to the infection. It is important to differentiate these two subsets of cCMV, symptomatic and asymptomatic infection. These are two different entities with different outcomes of the disease. Symptomatic cCMV infection can be a multisystem disease affecting several organs, or a more isolated disease with symptoms limited to only one end organ [164-168]. The apparent symptoms typical for cCMV infection are growth restriction, microcephaly, hepatosplenomegaly, jaundice, petechiae, and central nervous system (CNS) abnormalities [164-166, 168-170]. The skin may present with blue-red maculopapular lesions because of extra medullary hematopoiesis in the dermis [169, 171]. Pneumonitis is a rare manifestation of cCMV [167].

Imaging and laboratory testing may reveal thrombocytopenia, neutropenia, anemia, elevated liver enzymes, and CNS abnormalities like intracerebral calcifications, ventriculomegaly, cystic malformations, and neuronal migration abnormalities. On further evaluation, sensorineural hearing loss, chorioretinitis, optic atrophy, and retinal hemorrhage may be detected [164-166, 168-170]. The symptoms in cohorts of symptomatic cCMV are presented in Table 3.

Table 3. *Clinical findings of infants with symptomatic cCMV infection.*

Manifestation	Proportion	Reference
Prematurity	21%–50%	[164-166, 168]
Growth restriction	27%–50%	[164-168]
Microcephaly	20%–53%	[164-168]
Hepatosplenomegaly	22%–60%	[164-167]
Elevated transaminases	17%–83%	[164-166]
Jaundice	37%–67%	[164, 166, 167]
Conjugated hyperbilirubinemia	47%–69%	[164, 165]
Thrombocytopenia	50%–77%	[164-167]
Petechiae	45%–76%	[164, 165, 167]
Purpura	13%	[164]
Central nervous system abnormality	37%–68%	[164, 167]
Hearing loss	18%–59%	[165-168]
Chorioretinitis	14%–17%	[165, 167, 168]
Pneumonitis	7%	[167]

2.5.2 DIAGNOSIS OF CONGENITAL CMV

Diagnosis of cCMV infection in neonates is based on detecting the virus by culture, or parts of the virus genome by PCR-based methods, during the first 3 weeks of life. The rapid CMV urine culture is based on detecting CMV early nuclear antigen in urine by an immunofluorescence test [172]. CMV DNA can be detected in saliva or urine by real-time PCR [173, 174]. In research settings, the DBS collected from newborns for metabolic screening have been used for testing by real-time PCR for CMV. This testing has variable sensitivity depending on the assay and is not standardized for clinical use [175-178]. Perinatal and postnatal infections are very common and lead to CMV shedding in urine and saliva about 3 weeks after transmission. The positive samples collected after the age of 3 weeks cannot differentiate between the congenital and post/perinatal infections.

Serologic testing is not appropriate in diagnosing cCMV infection. During the last trimester, maternal IgG antibodies are transferred from the mother to the fetus, thus the IgG antibodies measured from infants reflect the antibody status of the mother. IgM antibodies are larger in size and are not transferred through placenta. The neonates, however, do not produce IgM antibodies in all cases of infection. The sensitivity of CMV IgM testing has been only 49%–71% in cCMV [179-181].

2.5.3 IMAGING IN CONGENITAL CMV

In order to evaluate the CNS findings of the cCMV, brain imaging is mandatory. Normal imaging findings predict good neurological outcome [167, 182]. Typical neuroimaging abnormalities due to cCMV infection include calcifications, cysts, ventriculomegaly, abnormalities in sulcation and gyration, delayed myelination, hypoplasia of corpus callosum, cerebellar abnormalities, and white matter abnormalities [167, 182-185].

Cerebral ultra sound (US) is an easy, non-invasive examination. It is suitable for screening of major abnormalities. It has good accuracy in detecting periventricular and parenchymal calcifications, cysts, and abnormalities in ventricle size. However, it does not detect migration or myelination abnormalities or white matter defects. Posterior fossa, cerebellum, and subtentoria spaces are also not well visualized with US. Minor non-specific abnormalities with ambiguous significance such as lenticulostriatal vasculopathy and germinolytic cysts can be detected with US. Computerized tomography (CT), on the other hand, has a good capacity for evaluating the gross structural abnormalities and calcifications. For a developing brain, however, the radiation exposure is high and CT is no longer recommended. Magnetic resonance imaging (MRI) has the best accuracy in finding the migration and white matter abnormalities but is not as sensitive in showing calcifications as US or CT [183-185].

2.5.4 OUTCOME OF CONGENITAL CMV

2.5.4.1 Hearing

SNHL is the most common long-term sequela of cCMV, affecting 9%–22% of all children with cCMV [59, 78, 86, 91, 92, 95, 186-189]. The pathogenesis of CMV-related hearing loss remains unclear. The virus and inflammation are present in the structures of inner ear [190, 191]. Virus has been identified in the stria vascularis, supporting cells of the organ of Corti, in the vestibule and non-sensory epithelium, as well as in the endolymphatic sac [190, 191]. These

structures regulate the endolymphatic secretion and potassium balance. It is hypothesized that abnormal potassium homeostasis may have a role in the pathogenesis of progressive and late-onset hearing loss in these children [190, 191].

According to the meta-analysis, hearing loss has appeared in 12.6% (95% CI 9.4–16.3) of cCMV infants identified from universal screening [192]. The impairment has been far more prevalent after symptomatic cCMV (32.8%, 95% CI 23.2–43.2) than asymptomatic cCMV (9.9%, 95% CI 6.3–14.2). In different cohorts of infants identified in screening, the prevalence of SNHL among symptomatic infants has varied from 22%–55% and among asymptomatic from 5%–21%, presented in Table 4 [59, 78, 86, 91, 92, 95, 186-189]. In cohorts of more selected patient groups such as referrals and infants diagnosed based on clinical symptoms, the proportion of hearing loss among symptomatic cCMV has been higher, up to 67% [193-195].

SNHL may be late onset, appearing during the first months or even years of life. Hearing loss can be also progressive or fluctuating. In a meta-analysis presenting data on cCMV-associated hearing loss among both screening and clinical cohorts, late-onset hearing losses constituted 18.1% of hearing losses in symptomatic and 9% in asymptomatic cCMV [192]. The majority of hearing losses detected in symptomatic children were bilateral (71.2%), in contrast to otherwise asymptomatic children with mostly unilateral hearing loss (56.9%) [192]. SNHL seems to be as common in the groups of babies infected due to maternal primary as non-primary infections [86, 188, 196].

Hearing loss is less common among the cohorts of children identified by screening, than in cohorts presenting data from clinical series. In screening, the mildly affected children with better prognosis are identified [197]. Also, the methods for assessing hearing loss and length of follow-up may result in different findings on hearing outcomes observed. In the earlier studies, before launching the universal hearing screening, the neonatal hearing data from otherwise asymptomatic infants was more likely to be unavailable, compared to the recent studies.

Table 4. *Screening studies presenting prevalence of sensorineural hearing loss among children with symptomatic (sympt) or asymptomatic (asympt) cCMV, listed in order according to the location of the study.*

Location	Follow-up time	Number of cCMV subjects	Proportion of cCMV children developing hearing loss in follow-up			Reference
		Sympt/ All	All	Sympt	Asympt	
USA	72 months	53/388 (13.7%)	15.4%	36.4%	11.3%	Fowler [186]
USA	34 months	18/76 (23.7%)	15.8%	44.4%	7%	Boppana [187]
USA	59 months	33/300 (11%)	10.7%	NA	NA	Ross [198]
USA	4 years	19/296 (6.4%)	8.7%	31.6%	7.2%	Ross [91]
Sweden	1-5 years	9/43 (20.9%)	9.3%	22%	5.9%	Harris [189]
Sweden	2-4 years	0/12	18.2%	-	18.2%	Engman [59]
Belgium	33 months	3/60 (5%)	21.7%	33%	21%	Foulon [86]
Japan	7 years	0/17	11.8%	-	11.8%	Numazaki [92]
Japan	NA	1/53 (1.9%)	9.4%	NA	NA	Yamaguchi [95]
Brazil	47 months	11/85 (12.9%)	11.8%	54.5%	5.3%	Yamamoto [78]

Sympt=symptomatic, asympt=asymptomatic, NA=not available

2.5.4.2 Neurology

Congenital CMV infection may lead to neurological abnormalities ranging from mild behavioral impairments such as attention deficit hyperreactivity disorder (ADHD) to severe intellectual disability, and motor deficits including cerebral palsy. There are at least three mechanisms that may cause the damage: uncontrolled viral replication in brain tissue causing damage, immunomediated damage, and placental infection leading to placental insufficiency and thus hypoxic brain damage [121].

In cohorts of symptomatic infants, the proportion of neurological abnormalities ranges from 25%–80% [96, 199, 200]. The proportion of sequelae in symptomatic infants has been lower in screening studies compared to the cohorts evaluating clinically diagnosed cCMV infants [197]. As discussed earlier, the children with only mild or isolated symptoms with better prognosis can easily be missed without screening.

The proportion of neurologic sequelae among asymptomatic children has been reported to be much lower, up to 14.5% [92]. Interestingly, many studies comparing neurological outcome of asymptomatic cCMV children and healthy controls did not find a difference between groups [194, 201-205]. Lopez et al observed no difference in academic achievements among the 89 asymptomatic cCMV children with normal hearing compared to 40 controls [206]. The follow-up was long (median 13 years), and the study comprised almost all of the infants originally identified in the screening (89/92). Another study by Pearl et al with a follow-up of 2 years did not find a difference in neurodevelopment assessed in 36 asymptomatic infants with cCMV and 74 controls [204]. In a study from China by Zhang et al, on the contrary, the 49 children with asymptomatic cCMV had significantly lower verbal and full-scale IQs compared to 50 healthy controls at 48 to 72 months of age [79]. The Chinese study was performed in an area with high incidence of intellectual disability; however, such disability was not more common among cCMV infants than controls [79]. Other studies comparing the outcome with controls had either a small number of participants or a high proportion lost to follow-up [92, 202, 203, 207]. In Ahlfors's study, the children with cCMV without any CNS symptoms at birth had significantly more abnormalities in neurodevelopment (18.3%, 11/60), compared to controls (2.6%, 1/39) evaluated at 7 years of age [88]. However, these children were not asymptomatic, since many of them had symptoms outside of CNS after birth. The neurologic outcome in cohorts of cCMV children identified in screening studies is presented in Table 5.

Table 5. *The neurologic outcome evaluated in the cohorts from cCMV screening studies.*

Location	Length of follow-up	Description of population	Results	Reference
USA	7.6 y (4.5-10.5)	17 asympt cCMV 21 controls	WISC score: no difference between cCMV and controls IQ: no difference between cCMV and controls Berde-Gestalt test: no difference between cCMV and controls	Kumar [202]
USA	Median 13 y for expressive vocabulary, and 17 y for other measures	78 asympt cCMV, normal hearing at age of 2 y 11 asympt cCMV, SNHL by age of 2 y 40 controls	Full scale intelligence score: no difference between asymptomatic cCMV with normal hearing and healthy controls. Asymptomatic cCMV with hearing loss had significantly lower scores than healthy controls. Verbal and nonverbal intelligence scores: no difference between groups Receptive vocabulary scores: cCMV with hearing loss had significantly lower scores than controls. No difference in scores between cCMV with normal hearing and controls. Expressive vocabulary scores: No difference between groups Academic achievements in maths or reading: no difference between groups	Lopez [206]
USA	6.5-12.5 y	18 asympt cCMV, no SNHL 18 controls	WISC: No difference between cCMV and controls K-ABC: No difference between cCMV and controls WRAT: No difference between cCMV and controls	Conboy [203]
UK	2 y	36 asympt cCMV 5 sympt cCMV 74 controls	Griffiths: No difference between asympt cCMV and controls. Sympt cCMV had significantly lower scores than controls.	Pearl [204]
Sweden	7 y	60 cCMV, no CNS symptoms at birth 39 controls	Stotts test / clinical evaluation: Abnormal Stotts test or mental retardation in 11/60 cCMV, abnormal Stotts test in 1/39 controls	Ahlfors [88]
Sweden	21 mo (Griffiths) 7 y (WISC)	35 cCMV with no CNS abnormalities or SNHL identified during the first year 53 controls	Griffiths: no difference between cCMV (n=32) or controls (n=51) WISC: no difference between cCMV (n=25) and controls (n=41)	Ivarsson [207]

Japan	6 y	32 asympt cCMV	WISC-III: IQ normal (>90) in 85.7% (18/21), IQ mildly impaired (70-89) in 14.3% (3/21) No major motor disorders	Numazaki [92]
Italy	2 y	2 sympt cCMV 14 asympt cCMV	Clinical evaluation: No clinical abnormalities at 2 years old	Barbi [62]
Japan	9-27 mo	59 asympt cCMV 1 sympt cCMV	Clinical evaluation: 6/60 abnormalities in follow-up: psychomotor development delay and speech delay in 4, psychomotor delay and hemiparesis in 1, and microcephaly and speech delay in 1.	Yamaguchi [95]
Japan	Mean 36 mo (15-60)	16 children with sympt cCMV	KSPD/ Clinical evaluation: Severe impairment 4/12, mild impairment 3/12, normal development 5/12	Nishida [96]
China	48-72 mo	49 asympt cCMV 50 controls	WPPSI: Verbal IQ and Full-scale IQ were significantly lower among cCMV than controls. Intellectual disability was as common in cCMV and controls.	Zhang [79]

asympt=asymptomatic, sympt=symptomatic, WISC=Wechsler Intelligence Scale for Children, K-ABC Kaufman Assessment Battery for Children, WRAT=Wide Range Achievement Test, IQ=Intelligence quotient, WPPSI=Weschler Pre-school and Primary Scale of Intelligence, KSPD=Kyoto Scale of Psychological Development, CNS=Central nervous system, mo=months, y=years

2.5.4.3 Ophthalmology

Visual impairment has been detected especially among symptomatic infants. In a cohort of 48 congenitally infected children, 39% (7/18) of symptomatic infants had a fundoscopic abnormality at neonatal period and 22% (4/18) had a visual impairment at a later follow-up visit at 16 to 72 months of age. None of the 30 asymptomatic infants had ophthalmologic findings either at neonatal or later examinations [208]. In another study by Jin et al, 26% (15/67) of symptomatic and 5% (5/95) of asymptomatic infants had visual impairments at follow-up. Only mild impairments appeared in the asymptomatic infants, as opposed to symptomatic infants with mostly severe impairments (10/15) [209]. In a long-term study by Lanzieri et al. evaluating only symptomatic infants, 52% (39/76) had ophthalmologic abnormalities [210]. Vision was normal in 67% (44/66), whereas 21% (14/66) were severely impaired or blind [210]. The most common ophthalmological abnormalities reported among cCMV patients were retinal scars, optic nerve hypoplasia, microphthalmia, strabismus, and cortical visual impairment [208-212].

2.5.4.4 Other problems related to congenital CMV

Other non-specific symptoms such as balance disturbances and feeding problems have been associated with cCMV infection as well [213]. In addition, tooth pathology, such as discoloration and enamel abnormalities, have been reported [214].

2.5.4.5 Outcome in maternal primary and non-primary infection

It had previously been assumed that maternal non-primary infections are less severe and seldom cause harm to the infant [215]. However, this finding has been questioned in several studies [198, 200, 208, 216-219]. Symptomatic cCMV seems to be equally common among children infected after maternal primary and non-primary infections, occurring in about 10% of infected infants [188, 219]. Similarly, the long-term sequelae are common in both groups [219]. Hearing loss developed on average in 11% of children after maternal primary infection and 14% after maternal non-primary infection [219]. Other neurodevelopmental abnormalities developed in 9% of children in primary infections in contrast to 39% in the non-primary group [88, 198, 216, 217, 219].

There are several studies where a large cohort of newborns has been screened for cCMV and the type of maternal infection has been categorized as either

primary or non-primary. The maternal non-primary infections accounted for 20%–93% of the pregnancies leading to cCMV [78, 85, 88, 113, 200]. In a Brazilian study, 93% (40/43) of cCMV infants were infected after maternal non-primary infection [216]. Hearing loss developed in a third (1/3) of infants infected after maternal primary infection in contrast to only 15% (6/40) of infants infected after maternal non-primary infection [216]. In London, only 20% (13/65) of maternal infections were non-primary [200]. Of the children infected after maternal non-primary infection, 15% (2/13) had moderate or severe abnormalities in long-term follow-up, in contrast to only 6% (3/52) in the primary group [200]. In a Swedish study of 76 cCMV infants identified in screening, the type of maternal infection could be determined to be primary in 48% (30/62) and non-primary in 52% (32/62) [88]. Neurologic abnormalities in follow-up until 7 years of age occurred in 19% (5/27) after primary infection, and 26% (6/23) after non-primary infection [88]. In a recent French study, nearly half (48%, 21/44) of cCMV children identified in screening had acquired infection after maternal non-primary infection [85]. Four children had SNHL identified after birth: two in children infected after maternal primary, and two after maternal non-primary infection [85]. In Greece, 90% (9/10) of cCMV children identified after screening of 2,149 neonates were infected due to maternal non-primary infection, all asymptomatic [113]. Two of the 5 children at follow-up had eventually developed hearing loss, both infected after maternal non-primary infection [113].

In the analysis of only symptomatic infants identified in screening in Birmingham, Alabama, maternal infection was either confirmed or presumed non-primary in 60% (12/20) of the cases [217]. Of the children with follow-up data, any long-term sequelae occurred in 75% (6/8) in the non-primary infection group: intellectual disability in 57% (4/7), motor abnormalities or chorioretinitis in 13% (1/8), and SNHL in 0/8 children. In children affected after maternal primary infection, 38% (3/8) had sequela: 13% (1/8) motor abnormality and 29% (2/7) SNHL [217]. In another study from Birmingham, Alabama, the hearing outcome was analysed in 300 cCMV children identified in screening with preconceptional and prenatal serum samples available for categorization of maternal infection [198]. Most children, 59% (176/300) were infected due maternal primary infection. Hearing loss was as common in both groups. SNHL occurred in 10% after maternal non-primary, and 11% after primary infection. However, the hearing loss was more often progressive and severe/profound in the primary infection group [198].

In an Italian cohort of cCMV referrals (diagnosed based on clinical symptoms or known maternal CMV infection), an impaired neurological outcome was observed in 24% of children in primary infection and 25% in non-primary infection. Similarly, hearing loss was equally common occurring in 26% in both groups [220].

2.5.4.6 Viral loads and outcome

The outcome of cCMV infection varies substantially, from asymptomatic infection to severe permanent damage. As presented earlier in this literature review, the presence of clinical symptoms at birth has been the major prognostic factor. In several studies, the measured viral loads have been higher among the symptomatic than asymptomatic children [181, 187, 221, 222]. The viremia level and length of viral excretion could theoretically be factors influencing the long-term outcome also among asymptomatic infants. One mechanism of CMV-induced pathology that has been hypothesized is the direct cytopathic effect of the ongoing viral infection. The higher levels and longer time for viral replication could explain a more massive cytotoxic effect.

However, studies have shown controversial findings. In a study by Forner et al, the viral load measured in the blood of asymptomatic infants was significantly higher among children who developed sequelae (mean 17,045 copies/ml) than children without sequelae (mean 1770 copies/ml) ($p<0.05$) [223]. In that study, the proportion of long-term sequelae was 30%, higher than in most studies among asymptomatic infants. All included children were born after maternal primary infection [223]. In another study by Zavattoni et al, however, the viremia level among 89 asymptomatic infants was not significantly correlated to the progression of long-term symptoms [181]. In a study from Alabama, the viral load measured during the first 2 months of life was not associated with hearing loss in symptomatic or asymptomatic children [221], nor was the viral load in CMV-positive DBS cards associated with development of SNHL [91]. These findings are discordant to the earlier partly overlapping but smaller cohort from Alabama by the same group, where the viral load in the urine and peripheral blood was associated with the development of SNHL in otherwise asymptomatic infants [187]. In that study by Boppana et al, however, no association of viral loads and SNHL was observed among symptomatic infants [187].

Forner et al did not find a significant difference in the length of viral excretion in blood or urine between the children with long-term sequelae and the children without sequelae [223]. Noyola et al reported that children with a shorter duration of viral shedding were more likely to develop hearing loss, in contrast to Rosenthal et al, who found an association between longer duration of excretion and development of hearing loss [224, 225].

In summary, according to the literature, the viral loads tend to be higher among symptomatic infants, but the level of CMV DNA measured in either symptomatic or asymptomatic infant is not a good single predictor of later sequelae at follow-up.

2.6 TREATMENT, PREVENTION, AND SCREENING FOR CONGENITAL CMV

2.6.1 TREATMENT OF CONGENITAL CMV

In 1997, Whitley et al published a non-randomised phase II treatment study of severely neurologically affected cCMV infants. The 6 weeks of intravenous ganciclovir treatment showed stabilization of hearing loss and better neurologic outcome in the follow-up compared to historical cohorts [226].

Later in a phase III study, by Kimberlin et al (2003), cCMV infants with CNS involvement were randomised to receive a 6-week course of intravenous (IV) ganciclovir or no treatment [227]. In the treatment group, none of the 25 babies in the ganciclovir group had a worsening in hearing at 6 months of age compared with 41% (7/17) of the non-treated babies. However, when the hearing was assessed again at the age of one year or older, 21% (5/24) in the ganciclovir group had developed further hearing impairment, compared to the baseline assessment. In the non-treated patients, as many as 68% (13/19) had hearing deterioration in the better ear from baseline to the second evaluation at the age of one year or older. None of the 19 non-treated babies and only 17% (4/24) of the treated babies had improved hearing in the second evaluation, compared to the baseline level [227]. The neurodevelopmental outcome was also better among the treated babies at the age of 6 months and 12 months [228].

Kimberlin et al's finding that the initial response of IV ganciclovir was favourable but later was diminished raised question about the length of the treatment. Was 6 weeks enough? Could a longer treatment better prevent further deterioration of the hearing? Amir et al later published retrospective data on 23 patients treated with a longer course of antivirals in Schneider Children's Medical Center in Israel. All their patients had CNS affision before therapy and were treated with a 12-month protocol: 6 weeks IV ganciclovir followed by oral valganciclovir until the age of 12 months [229]. The full 12-month treatment was finished by 19 of 23 infants. The authors compared the hearing results with the previous cohort of Kimberlin [227] with a 6-week treatment with IV ganciclovir. The selection for treatment and follow-up regimen were similar in the cohorts. The hearing outcome at the age of one year or older was better in Amir's cohort of longer treatment (p=0.001) [229].

The question of short versus long treatment protocols were investigated in a large randomised controlled trial (RCT) published in 2015 [230]. Kimberlin et al compared a 6-week treatment to a treatment of 6 months with oral valganciclovir. There was no difference in the occurrence of adverse effects between the groups. The hearing outcome was equal after the 6-month follow-

up, but modest benefit in the longer treatment group compared to shorter treatment was noticed in the 12-month and 24-month hearing follow-ups. The significant difference was observed when the number of children with either normal or improved hearing was compared to the number of children with either worsened hearing or the same degree of hearing loss as at baseline. The difference was statistically significant only in the analysis adjusted for CNS involvement at baseline. It has to be noted, that despite randomisation, there was some bias in the baseline characteristics of the treatment groups, which may influence the results. At baseline before treatment, 74% (32/43) of the 6-month therapy group had normal hearing in the best ear, compared to only 58% (25/43) of the 6-week treatment group. Only 7% (3/43) of children in the 6-month treatment group had severe hearing loss at baseline, in contrast to 19% (8/43) in the 6-week therapy group [230].

The children in the 6-month group had significantly better outcomes in the language composite and receptive-communication scale in the Bailey-III scales at 24 months of age. That difference was seen in the groups of children with and without baseline CNS affision [230].

Ganciclovir and valganciclovir are potentially toxic drugs inducing neutropenia in a notable proportion of treated patients [226, 227, 229, 230]. In animal models, there have been worries about ganciclovir having gonadotoxic effects. The effects have not been demonstrated in humans. Rodents exposed to ganciclovir in utero or treated with high-dose ganciclovir have shown abnormal testicular size, histology, and impaired spermatogenesis [231-234].

Based on the current evidence of limited benefits from antiviral treatment and potential severe adverse effects, the guidelines suggest offering antiviral treatment only to cCMV children with moderate to severe symptoms [235, 236].

2.6.2 PREVENTION OF CONGENITAL CMV

Since the therapeutic options to prevent CMV-related disabilities are limited, measures to prevent maternal infection during pregnancy have been essential. The prevention of primary CMV infections by hygienic measures have been evaluated in several studies [237-239]. These measures included washing hands after exposure to children's excretions, avoiding saliva contact with children, such as kissing on the mouth, sharing utensils, washcloths, and food and drink, and not sleeping in the same bed with a child. In a study by Adler et al, the seroconversion rates were examined among women pregnant or attempting pregnancy who had a young child attending daycare [237]. The seroconversion rate was same in the intervention group receiving information

on how to prevent child-to-mother transmission as in the control group, 7.8% (9/115) and 7.8 (5/51), respectively [237]. In another study by Valoup-Fellous et al, 5,173 pregnant women were tested for CMV antibodies at around 12 weeks gestation [238]. Seronegative pregnant women were counselled for CMV and preventive measures. Based on interpreting avidity testing, primary infection had occurred in 0.42% of women in early pregnancy before 12 weeks gestation. However, seroconversion occurred in only 0.19% (5/2,583) of seronegative mothers after 12 weeks gestation. The authors conclude that the counselling after known seronegativity could have reduced the seroconversions in later pregnancy [238]. Revello et al evaluated the seroconversion rates among seronegative pregnant women who were counselled for CMV and preventive measures at 12 weeks gestation [239]. The seroconversion rates were compared to those of women who were not counselled but whose early pregnancy samples were available for assessing retrospectively whether seroconversion had occurred between 12 gestational weeks and delivery [239]. Seroconversion was observed in 1.2% (4/331) in the intervention group and 7.6% (24/315) in the control group ($p<0.001$), demonstrating that the intervention of sharing information on CMV and on prevention of transmission was efficient in reducing new infections [239].

Vaccines to prevent CMV infections have been under development for more than 50 years without success. The main goal in vaccine development has been the prevention of cCMV infections, but other aims such as preventing CMV-induced complications among transplant patients also exist [46]. However, vaccine development is complicated by the fact that natural immunity does not give protection for recurrent infections and fetal disease. The risk for materno-fetal transmission, however, is reduced by the existing maternal antibodies. The goal could be to prevent primary infections among pregnant women. In addition, through herd immunity the total burden of CMV in the community could be diminished, thus reducing potential CMV exposure among pregnant women [46].

Administering CMV hyperimmunoglobulin (CMV-HIG) to reduce the risk of fetal infection or to diminish the symptoms of infected infants in cases of maternal primary infection during pregnancy has been investigated. In 2005, Nigro et al published a non-randomised prospective study where CMV-HIG was offered to women with primary CMV infection during pregnancy. The transmission risk in the HIG group was lower (16%) than that in non-treated mothers (40%) [240]. Later in a case-control study, the same author retrospectively compared the factors of cCMV children with sequelae and cCMV children without sequelae. HIG treatment was an independent predictor of better outcome [241]. A retrospective study evaluated the pregnancies treated with off-label CMV-HIG in four countries in Europe from 2006 to 2010 [242]. The vertical transmission rate was 20.8% after primary infection in the first trimester and 26.6% after primary infection in the second

trimester. These transmission rates were lower than in most earlier published cohorts. The variation in the previous studies, however, has been wide as well: 36% (22.2%–42.2%) after the first trimester and 40.1% (26.9%–44.9%) after the second trimester [108]. In a prospective study, CMV-HIG was offered to mothers with primary infection early in the pregnancy, and the outcome was compared to pregnancies without HIG treatment. The non-treated pregnancies mainly occurred before the HIG protocol had started [243]. The outcome in children of the treated mothers was better. At 1-year follow-up, an adverse outcome was observed in 43% (16/37) of infants born to untreated mothers compared to only 13% (4/31) of treated mothers [243].

However, the major problem in all these non-randomised studies has been the selection bias of treated/non-treated women. The first and only randomised trial of CMV-HIG was published in 2014 by Revello et al [244]. It showed no benefit of the treatment. Adverse outcomes were significantly more common in the pregnancies after HIG treatment [244]. Women (n=124) with primary CMV infection at 5 to 26 weeks gestation were randomised to receive HIG treatment (n=61) or placebo (n=63) every 4 weeks until 36 weeks gestation. In the treatment group, 30% (18/61) were infected, and in the placebo group, 44% (27/62) of fetuses were infected. The difference was not statistically significant. However, the proportion of obstetrical complications (e.g., prematurity, preeclampsia, fetal growth restriction) was significantly higher in the HIG group (13%, 7/53) than in the placebo group (2%, 1/51) (p=0.06) [244]. In summary, there is no good evidence to support the usage of HIG to prevent vertical transmission.

2.6.3 SCREENING FOR CONGENITAL CMV

Most children with cCMV are asymptomatic. Therefore, testing only children with suspected CMV infection finds only a small proportion of all infected infants [197, 245]. Universal screening would enable detection of all affected infants. Testing mothers for CMV antibodies in early pregnancy enables identification of seronegative mothers at risk for CMV primary infection during pregnancy. Furthermore, serial testing will reveal new seroconversions. However, serological screening of mothers fails to identify the infants at risk of infection due to maternal non-primary infections. Two strategies for neonatal screening have been used: universal screening and targeted screening. In targeted screening, the children who fail the newborn hearing screening are tested for CMV. In universal screening, all children are tested. Targeted screening is no doubt less expensive compared to universal screening. It, however, identifies only those children with CMV-related early onset hearing loss and fails to recognize those children who develop hearing impairments later in the infancy [247].

The early identification of all infected infants would allow early interventions. On one hand, selected infants could benefit from antiviral therapy. On the other hand, the early diagnosis would improve the possibilities to start rehabilitation early, in case of hearing loss, visual abnormalities, or neurological problems associated with cCMV [248, 249].

Universal hearing screening already detects the cCMV children with early onset hearing loss, and screening for cCMV would not give an extra advantage. However, the children who develop late-onset hearing loss soon after birth would benefit from the early diagnosis. In an analysis comparing children born before and after universal hearing screening, the early detection of bilateral hearing loss before 9 months of age was associated with significantly better language abilities [248, 250].

As regards to neurologic abnormalities, the early diagnosis enables more careful follow-up of the affected infants, and early rehabilitation could be offered in case of developmental abnormalities. Nevertheless, the developmental and cognitive abnormalities among asymptomatic infants are only slightly more common than in the general population. In Finland, all children undergo regular developmental assessments according to national guidelines in the primary care and rehabilitation is provided if needed. Thus, an early diagnosis of asymptomatic CMV cases might not have a major effect on later developmental problems. However, one potential benefit of screening is that the time window for reliable diagnosis is during the early weeks of life only. If the delayed development is noticed later, when peri- or postnatal infection may have occurred, congenital infection cannot be excluded by any laboratory method. In these cases, the diagnosis of cCMV infection in screening would be helpful for families, and some other unnecessary ethiological testing might be avoided.

There is evidence of antiviral medication leading to better hearing and neurocognitive prognosis in symptomatic children with cCMV [227, 228, 230]. Symptomatic infection can be identified clinically without screening. There is no data, however, supporting the treatment of asymptomatic infants with antivirals. The early diagnosis, with the current evidence, would not lead to interventions preventing the development of late-onset hearing loss or impaired neurological outcome among asymptomatic infants.

There are several aspects to questioning universal screening. First of all, it would potentially cause anxiety in many families. This is an important issue, especially since there is no effective intervention to prevent long-term sequelae among asymptomatic infants. Second, a vast majority of these asymptomatic infants heal without any sequelae.

The cost-benefit analysis of both targeted screening and universal screening has indicated some cost savings in the American healthcare system. However, since the evidence of potential long-term benefits of antiviral treatment is not strong, these analyses contain many assumptions [251, 252].

3 AIMS OF THE STUDY

The aim of the study was to evaluate the disease burden of cCMV infection in Finland (Figure 3). The specific aims of the study were to:

1. Evaluate the CMV seroprevalence among Finnish pregnant women and analyse the changes in seroprevalence during recent decades (I)

2. Evaluate the prevalence and outcome of asymptomatic cCMV infection in the Helsinki area, among children diagnosed from screening (III)

3. Evaluate the long-term outcome of symptomatic cCMV infection diagnosed in Finnish University Hospitals in 2002–2012 (II)

4. Evaluate the type of maternal infection (primary / non-primary) in the pregnancies leading to symptomatic or asymptomatic cCMV infection (II, III)

5. Describe the subsequent viral shedding in urine, saliva, and plasma, and describe the patterns of CMV gH, gB, and gN genotype distribution among congenitally infected infants identified in screening (IV)

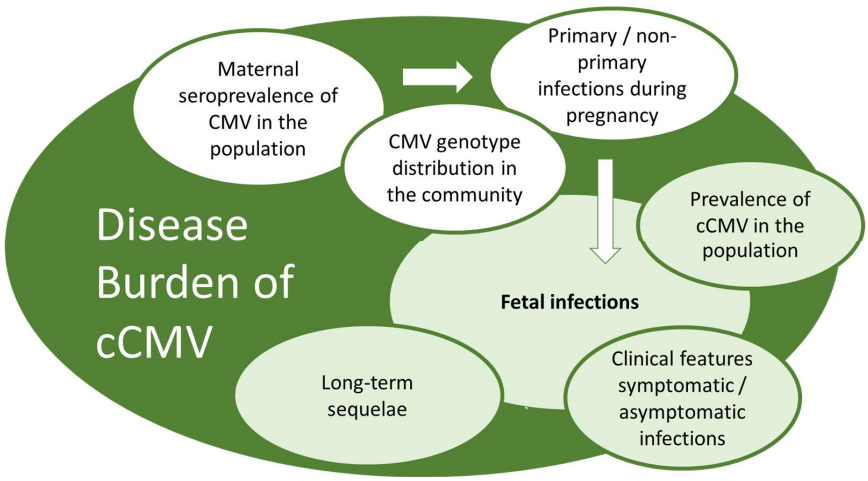

Figure 3 The aim of the study was to evaluate the disease burden on cCMV in Finland. Disease burden is a multifactiorial entity consisting of features in the population, hosts, and pathogens.

Table 6. *The thesis consists of Studies I-IV presented in the table.*

	Database/ Register	Study years	Study design	Study setting	Population	Tests	Main research question
Study I	Finnish Maternity Cohort Serum Bank	1992, 2002, 2012	Cross sectional study	Finland, all areas	200 pregnant women / year	CMV IgG	Changes in seroprevalence for CMV among pregnant women during recent decades
Study II	Clinical patient data files	Children born 2000-2012	Retrospective study	University Hospitals in Finland: Helsinki, Turku, Tampere, Oulu, and Kuopio	26 children diagnosed with symptomatic congenital CMV infection	Clinical follow-up data on neurology, hearing, and ophthalmology	Long-term outcome of children with symptomatic congenital CMV infection
Study III	Prospective screening	Children born 2012-2015	Prospective study	Four delivery hospitals in Helsinki area	19,868 infants screened for congenital CMV. 40 CMV positive and 54 healthy controls followed up until age 18 months	Screening: Saliva CMV PCR Follow-up: Neurology Griffiths Mental Development Scales Hearing TEOAE, SF Ophthalmologic examination	Prevalence of congenital CMV infection and outcome of the cCMV-positive children identified in screening

Study IV	Prospective screening	Children born 2012-2015	Observational study	Four delivery hospitals in Helsinki area	40 children with congenital CMV identified in screening	Viral shedding: urine CMV culture, plasma and saliva CMV-PCR Genotypes: Genotype specific real-time PCR for gB and gH, PCR and cloning for gN	Viral shedding and distribution of CMV gH, gB, and gN genotypes in cCMV

TEOAE=Transient evoked otoacoustic emission

SF=Sound field audiometry

4 MATERIALS AND METHODS

4.1 PATIENTS AND POPULATION

Study I
The CMV seroprevalence was evaluated from serum samples from the Finnish Maternity Cohort (FMC) serum bank. The maternal sera were originally drawn for the purposes of screening for HIV, syphilis, and hepatitis B at the end of the first trimester in routine antenatal care. After informed consent, the leftover sera were stored in a national serum bank at the National Institute of Health and Welfare, for later research purposes. The bank contains about 90% of the antenatal sera sampled in Finland. For our study, 200 samples per time point (1992, 2002, and 2012) were randomly selected using SPP statistical software.

Study II
In study II, we retrospectively reviewed the patient data from children diagnosed with cCMV infection from 2000 to 2012. The patients were identified through a database search of all University Hospitals in Finland (Helsinki, Tampere, Turku, Oulu, and Kuopio) after searching for International Classification of Diseases, 10th Revision (ICD-10) code P35.1, cCMV infection. We included in the analysis only children with confirmed symptomatic cCMV infection with viral detection in either urine, blood, or saliva within the first 3 weeks of life and an available FMC serum bank sample. Symptomatic infection was defined as the presence of at least one CMV-related symptom: intrauterine growth restriction (<-2SD), microcephaly (<-2SD), thrombocytopenia (<80 x 10^9/l), petechiae, hepatomegaly or splenomegaly, calcifications in brain ultrasound, hepatitis (alanine aminotransferase >100U/l), or SNHL. A serum bank sample was available for 26 of the 29 children diagnosed with symptomatic cCMV infection during the time period, and these 26 children were included in the study.

Studies III and IV
We performed universal neonatal screening for cCMV infection in Helsinki area hospitals (Naistenklinikka, Kätilöopisto, Jorvi Hospital, and Lohja Hospital) from September 2012 to January 2015 to identify cCMV infants. The screening was offered to all infants whose parents understood either Finnish, Swedish, or English in order to read the study information leaflet.

The children who tested positive in the CMV screening were followed prospectively. For all positive children, one healthy control was selected, and the controls were matched according to the delivery date, gestational weeks at delivery, sex, and neonatal care unit.

4.2 METHODS

4.2.1 ANTIBODY ASSAYS (I, II, AND III)

Cytomegalovirus antibodies were measured from the FMC serum samples and the antenatal serum samples of mothers of CMV-positive infants to evaluate the type of maternal CMV infection, as presented in Table 7. In Studies I, II, and III, IgG was measured with commercial assay Architect CMV IgG Reagent Kit, Abbott Laboratories, Wiesbaden, Germany. In Studies II and III additionally, IgM and IgG avidity were measured with commercial Architect CMV IgM and Architect CMV IgG avidity assay. The avidity under 50% was considered low and over 60% was high according to the manufacturer's instructions. Avidities between 50% and 60% were considered a grey area with unreliable interpretation [253, 254]. The FMC samples (Study I and II) were tested during a short time frame per study, after receiving the archived samples. All samples were tested separately. The antibody assays for the prospective screening study (III) were performed whenever a positive infant was identified during the 2.5 years screening period.

Table 7. *Type of maternal CMV infection: interpretation of antenatal antibody results measured at the end of the first trimester.*

	IgG	IgM
Non-primary	Positive, high avidity	Positive or negative
1st trimester or near conception	Positive, low avidity	Positive
	Negative	Positive
2nd-3rd trimester	Negative	Negative

4.2.2 SALIVA SAMPLES (III, IV)

Screening saliva samples were collected from newborns born in Helsinki area hospitals from September 2012 to January 2015 during the first week of life. Follow-up samples from screening positives were collected at 3 months and 18 months of age. After informed consent was received from parents, a Dacron swab was placed in infant's mouth and removed after saturation with saliva. The swab was dried at room temperature, and dry swabs were stored at -20°C

until shipped to the University of Alabama, Birmingham (UAB), USA, for PCR analysis.

4.2.2.1 CMV real-time PCR (III, IV)

PCR analysis was performed in UAB, with a PCR test that was developed to diagnose CMV infection in neonates [173, 176]. The ABI 7500 Real-time PCR system (Applied Biosystems Inc., Foster City, CA) and Absolute™ QPCR Low ROX Mix (ABgene USA, Rockford, IL) were used. Primers to detect the highly conserved immediate early 2 exon 5 region were used. The sample was considered positive if one or more genomic equivalents per reaction were detected.

4.2.2.2 Genotyping for gB (UL55), gH (UL75), and gN (UL73) (IV)

CMV-positive screening saliva samples were further analysed for viral gB, gH, and gN genotypes. The genotype analysis was performed in UAB. For screening and follow-up samples, the evaluation for gB and gH was performed. Glycoprotein B and gH genotype-specific real-time PCR with the Taqman platform was used [32, 255, 256]. The CMV-positive screening saliva samples were also examined for genotype of gN. PCR was used to amplify the gN gene. PCR products were cloned using the TOPO TA cloning kit (Thermo Fisher Scientific), and the colonies were screened for the presence of the gN insert. The nucleotide sequences were compared with the previously published gN subtype sequences (GeneBank accession numbers AF309971, AF309976, AF309980, AF390773, AF309987, AF309997, AF310004) [41]. The nucleotide sequences were aligned using the Bio Edit software [15, 257].

4.2.3 URINE CMV CULTURE AND PLASMA CMV PCR (III, IV)

In the prospective screening study, urine and plasma samples were collected from screening positive infants at 3 months and 18 months of age. In-house rapid culture method was used to test for detection of early CMV nuclear antigen by immunofluorescence test in the urine. The plasma sample was tested for viral load by commercial assay (COBAS AmpliPrep/COBAS Taqman CMV test, Roche) according to the manufacturer's instructions. The analytical sensitivity of the assay is 56 IU/ml.

4.2.4 OUTCOME (II, III)

In the retrospective study of children with symptomatic cCMV, the outcome data were collected retrospectively from the patient records (Study II). In the

prospective screening study, data were collected prospectively in appointments at 3 months and 18 months of age. The same pediatrician evaluated all the subjects during the visits (Study III).

4.2.4.1 Retrospective data collection (II)

The outcome of children with symptomatic CMV infection (Study II) was evaluated based on clinical patient data files. Based on the clinical records, we assessed the outcome on neurology, hearing, and ophthalmology. Neurological outcome was defined as normal, mild abnormality, or severe abnormality. The neurology was considered normal if the neurological performance at the latest follow-up visit was appropriate for the age. Mild abnormalities included learning difficulties, problems with attention, and mild motor disturbances with normal communication abilities. Severe neurologic abnormalities included cerebral palsy, intellectual disability, and death. Hearing and ophthalmological outcomes were evaluated from the last clinical follow-up visit.

4.2.4.2 Prospective data collection (III)

Neurodevelopmental outcome was assessed using Griffiths Mental Development Scales, 1996 Revision, from birth to 2 years [258]. These scales evaluate the development in five distinct areas: A locomotor, B personal-social, C hearing and language, D eye-hand coordination, and E performance. According to the test manual, the subquotient for each developmental section and general quotient measuring the total development were determined.

An experienced audiologist evaluated the hearing of CMV-positive children and healthy controls. Testing was performed at 3 months and 18 months of age with TEOAE. It evaluates the acoustic response to a sound stimulus produced by the inner ear. It tests both ears separately and can reveal unilateral hearing losses. At 18 months of age, sound field (SF) audiometry was performed. SF measures the behavioral response to frequency-specific stimulus. It tests both ears simultaneously and cannot reveal unilateral hearing loss. However, it gives a good assessment of functional hearing capacity.

An ophthalmologist examined the eyes of subjects at 3 months and 18 months of age. Examination included fix and follow, contact and smiling, Hirschberg, convergence, cover test, red reflex, and refraction. The fundus was evaluated with indirect ophthalmoscope, including evaluation of optic nerve head, fovea, and midperiphery.

4.2.4.3 Fetal growth (II, III)

Intrauterine growth was evaluated from the birth measurements (i.e., weight, height, head circumference), according to the national reference values [259].

4.2.5 STATISTICS

Stata versions 11.1 and 14.2 (StataCorp LP, College Station, TX) and IBM SPSS Statistics version 22 (SPSS Inc., Chicago, IL) were used to perform statistical analysis. Fisher's exact or Pearson's chi-squared test (χ^2) were used to compare categorical variables. Parametric linear variables were compared with the *t* test and non-parametric linear variables with the Mann-Whitney U test. The proportions and prevalences were presented with 95% confidence intervals.

4.2.6 ETHICAL CONSIDERATIONS

The study protocols were approved by the coordinating ethics committee (I, II), and ethics committee for women, children, and psychiatry (III, IV) in the Hospital District of Helsinki and Uusimaa. In addition, the National Institute for Health and Welfare approved the data collection from patient files in study II. All studies were conducted in accordance with the guidelines of the Declaration of Helsinki. In Studies I and II, CMV antibodies were evaluated from the archived early pregnancy samples from FMC serum bank, collected after obtaining informed consent. The FMC steering group approved the study protocols (I, II). A written, informed consent was obtained from the parents before collecting CMV screening saliva samples from the infants (III, IV). The CMV-positive infants and healthy controls were followed up according to the protocol, enabling the early interventions such as hearing rehabilitation or physiotherapy, if needed (III).

5 RESULTS

5.1 MATERNAL SEROPREVALENCE OF CMV ANTIBODIES IN FINLAND (I)

The changes of seroprevalence for CMV among Finnish pregnant women from 1992 to 2012 are presented in Figure 4.

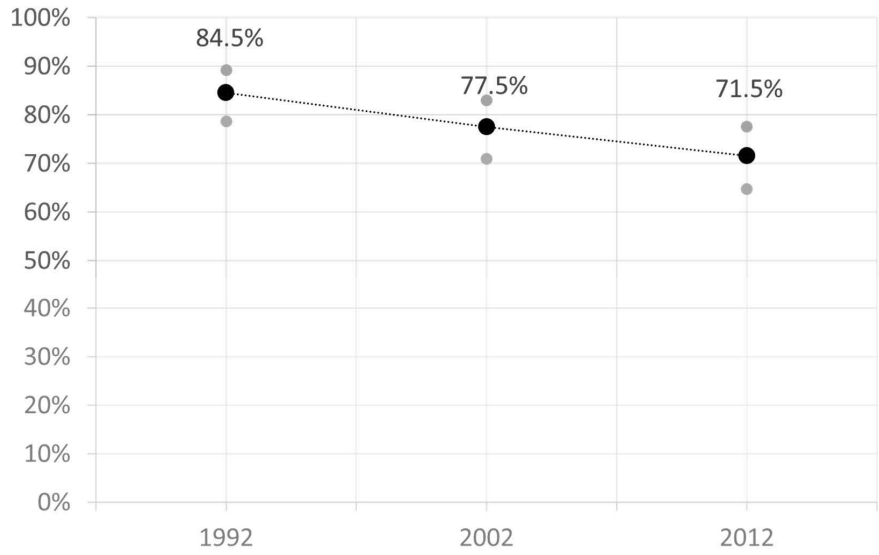

Figure 4 The seroprevalence for CMV in 1992, 2002, and 2012 among Finnish pregnant women. 200 samples per time-point were evaluated. CMV seroprevalence decreased from 84.5% (95% CI 78.7–89.2) to 71.5% (95% CI 64.7–77.6) between 1992 and 2012. Change was statistically significant, calculated by Fisher's exact test (p=0.007).

5.2 MATERNAL PRIMARY AND NON-PRIMARY INFECTIONS AND CONGENITAL CMV INFECTION (II, III)

The type of maternal CMV infections during pregnancy leading to birth of congenitally infected children in the studied retrospective and prospective cohorts are presented in Figure 5. The antenatal serum sample drawn at the end of first trimester was available for testing in 26 symptomatic cCMV children in the retrospective cohort and 38 children in the prospective

screening cohort. In both cohorts, the maternal non-primary infections dominate. The proportion of maternal primary infection in early pregnancy was higher in the retrospective cohort of symptomatic children (27%) compared to the screening cohort (16%); however, the difference was not statistically significant (p=0.277).

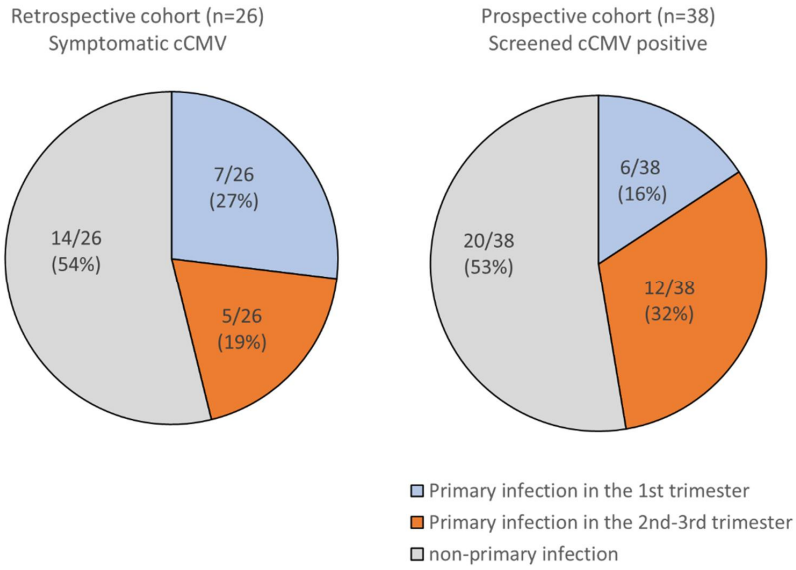

Figure 5 Proportions of maternal primary and non-primary infections in pregnancies leading to congenital CMV in the studied retrospective and prospective cohorts.

5.3 PREVALENCE OF CONGENITAL CMV INFECTION IN FINLAND (III)

5.3.1 POPULATION IN SCREENING

A total of 19,868 neonates were screened for cCMV infection during the study period of September 2012 to January 2015. Most screening samples were collected in the well-baby nurseries (98.3%) and minority in the basic neonatal wards (1.7%). In addition, 70 samples were collected in the neonatal intensive care unit (NICU). The sampling in the NICU was not representative due to low yield of samples, and it is not included in the analysis. Approximately 54% of infants born in the four study hospitals were screened. The screening percentages in each hospital during the study period are presented in Figure 6.

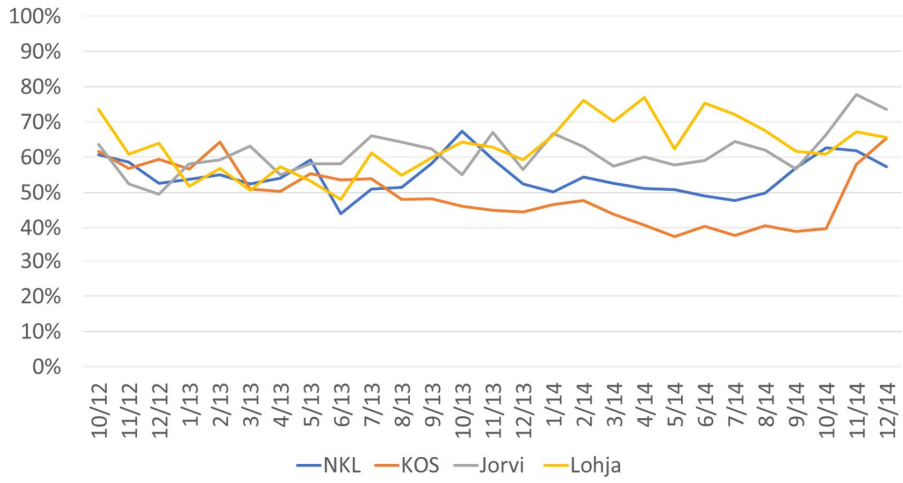

Figure 6 The proportion (%) of CMV screened infants of all infants born in the four screening hospitals (NKL=Naistenklinikka, KOS=Kätilöopisto Hospital, Jorvi Hospital, Lohja Hospital)

The demographic data were evaluated from the 9,167 mothers of screened infants who attended the screening between October 1, 2012, and October 31, 2013. The data and comparison to the general population in Finland are presented in Table 8 [260].

Table 8. *Demographics of mothers of CMV-screened infants versus the population in Finland.*

Characteristics	Mothers of screened infants[a]	General population
Born in Finland, %	88.1	89.8[b]
Born abroad, %	11.9	10.2[b]
Europe, %	7.3	6.3[b]
America, %	0.6	0.4[b]
Africa, %	1.4	0.9[b]
Asia, %	2.6	2.5[b]
Australia or New Zealand, %	0.03	0.03[b]
Primipara, %	38	41[c]
Mothers with ≥4 previous deliveries, %	1.6	4.8[c]
Number of children in the family, mean	1.86	1.75[d]
Married, %	66	58[c]

[a]Evaluated from 9,167 mothers screened between October 1, 2012, and October 31, 2013

[b]Women aged 20-39 years living in Finland, 2013 (n=822,321) [260]

[c]Live births in Finland, 2013 (n=58,134) [260]

[d]Total fertility rate, 2013 [260]

5.3.2 PREVALENCE OF CCMV

From 19,868 screening samples, 56 were positive. One screening positive infant did not attend any further follow-up and no control samples were collected. After control urine and saliva samples at age 3 months, 15 screening positive samples proved to be false positives. In total, 40 infants had confirmed cCMV infection, making the prevalence 2 out of 1,000 newborns (95% CI 1.4–2.6 out of 1,000).

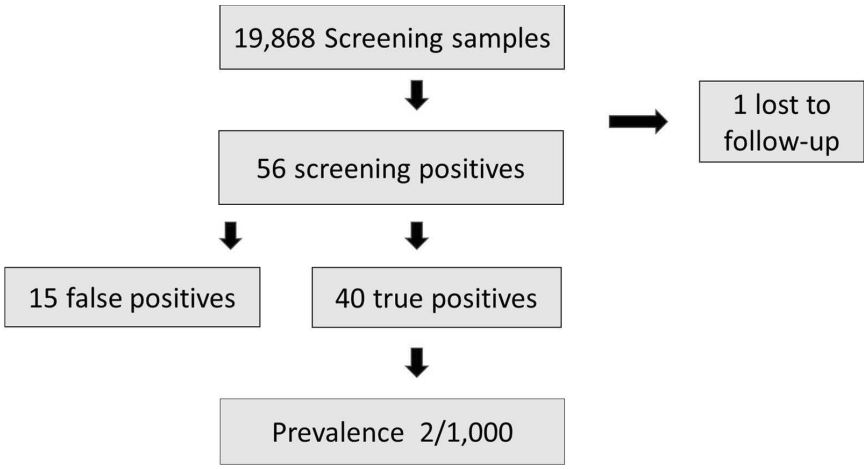

Figure 7 Flowchart of the congenital CMV screening. After screening of 19,868 neonates, the prevalence of cCMV was 2/1,000 (95% CI 1.4–2.6 out of 1,000)

The calculated prevalence in subpopulations according to mother's country of birth is presented in Table 9. We estimated the total number of screened populations per continent based on the data from 9,167 screened infants between October 1, 2012, to October 31, 2013. Due to low numbers, the 95% confidence intervals are wide.

Table 9. *Calculated prevalence of congenital CMV in Finland according to mother's country of birth.*

Mother's country of birth	Positive cCMV in all 19,868 screened	Prevalence/ 1,000[a]	95% CI[b]
All	40	2.0	1.4–2.6
Finland	34	1.9	1.3–2.7
Europe, not Finland	2	1.4	0.2–5.0
Asia	2	3.8	0.5–13.8
Africa	1	3.5	0.1–19.6
America	1	8.7	0.2–47.5
Australia and New Zealand	0	–	–

[a]Calculated based on data from 9,167 mothers screened between October 1, 2012, and October 31, 2013

[b]Binomial exact

5.3.2.1 False positives

Fifteen positive screening samples were regarded as false positives. Urine CMV culture was negative at age 3 months in 14 children. Congenital infection leads to prolonged CMV excretion in urine and is known to still be positive at 3 months [92, 261]. In the case of one false negative child, the 3-month urine sample was not collected.

In 12 children, false positivity was confirmed. Four children had no CMV antibodies at 3 months of age. In infants, the antibody status represents maternal antibodies and the mothers of these children did not have antibodies for CMV, thus also excluding the cCMV infection. Eight children, including one child with no urine sample collection at 3 months were negative for CMV antibodies at age 12 to 18 months. At this age, maternal antibodies had faded, and lack of CMV antibodies in the children excluded congenital infection.

In three cases, false positivity was not confirmed, and they are regarded as suspected false positives. In two cases with negative urine culture at 3 months, no confirmatory antibody sample was tested after fading of maternal antibodies. One child with negative urine culture and negative saliva CMV PCR at age 3 months had a positive urine culture and positive CMV antibodies at 18 months. She is considered as suspected postnatal infection. The laboratory examinations of the false positive children are presented in Table 10.

Table 10. *Test results at 3 months and 12–18 months of age from the 15 children with false positive congenital CMV screening samples. NT=not tested.*

Case	At 3 months			At 12–18 months	Interpretation
	Urine CMV culture	Saliva CMV PCR	CMV-IgG	CMV-IgG	
1	-	+	+	-	Confirmed false positive
2	-	-	+	-	
3	-	-	-	NT	
4	-	-	+	-	
5	NT	+	+	-	
6	-	-	+	-	
7	-	-	-	NT	
8	-	-	+	-	
9	-	-	+	-	
10	-	-	-	NT	
11	-	NT	-	NT	
12	-	+	+	-	
13	-	NT	+	NT	Suspected false positive
14	-	NT	NT	NT	
15	-	-	+	+	Suspected false positive and postnatally acquired infection

5.4 OUTCOME OF CONGENITAL CMV INFECTION (II, III)

5.4.1 OUTCOME OF SYMPTOMATIC CMV INFECTION (II)

5.4.1.1 Neonatal presentation

The symptomatic cohort consisted of 26 children diagnosed with cCMV infection in all university hospitals from 2000 to 2012. Mean gestational age at birth was 37 +2 weeks (ranging from 29 +5 to 41 +4 weeks). The majority of infants (18/26) were born at term, and eight of them were born prematurely (ranging from 29+5 to 35+4 weeks). Growth restriction was common, 58% (15/26) had a birth weight less than -2.0 SD, and 28% (7/25) had a birth height less than -2.0 SD. Microcephaly (head circumference less than -2SD) was found in 48% (11/23). Other symptoms during the neonatal period according

to patient files were petechiae (n=9), thrombocytopenia (n=13), anemia (n=3), neutropenia (n=2), leukopenia (n=2) hepato-/splenomegalia (n=12), elevated transaminases or icterus (n=4), and hypotonia or abnormal electroencephalogram (n=5).

5.4.1.2 Imaging

Brain ultrasound was performed for 23 infants during the neonatal period and was abnormal in 61% (14/23). Cerebral MRI was performed for 13 infants during the first year of life and was abnormal in 9 cases. Abnormal imaging findings are presented in Table 11.

Table 11. *Abnormal imaging findings in retrospective cohort of symptomatic congenital CMV. (II)*

Brain ultrasound n=23	Cerebral MRI n=13
Cysts or cystic abnormalities (n=8) • caudothalamic sulcus • periventricular • lateral ventricles • midline and occipitofrontal plexus Calcifications (n=9) • periventricular • parenchymal • thalamic • basal ganglia Dilated ventricles (n=6) Dilated cortical liquor space (n=1) Abnormal gyration (n=1) Candle stick abnormalities in thalamus (n=1) Lenticulostriatal vasculopathy (n=1)	White matter abnormalities (n=5) • signal intensification Cystic abnormalities (n=2) Calcifications (n=5) • periventricular • cortical • cerebellar Dilated ventricles, thin parenchyma in occipital and parietal lobes (n=1) Abnormal gyration (n=2) • cerebral • cerebellar Delayed myelinization (n=2)

5.4.1.3 Long-term sequelae

Based on the clinical files, 58% (15/26) of retrospectively evaluated symptomatic children had some long-term disability suspected to be caused

by the cCMV infection. Of the children whose mothers had had primary CMV infections in the early pregnancy, 86% (6/7) had some long-term sequelae. None of the 5 children whose mother had a primary infection in later pregnancy developed any long-term sequelae. Of the children whose mother had suffered from non-primary infection during pregnancy, 64% (9/14) developed long-term sequelae.

The neurological outcome could be evaluated from clinical files in 24 children. The average follow-up time was 21.7 months (ranging from 3 to 132 months). One premature child born at gestational week 30+3 with CNS lesions, and respiratory distress syndrome, died at the age of 3 days. Neurologic outcome was abnormal in half (50%, 12/24) of children. Severe disabilities appeared in 29% (7/24), and mild disabilities in 21% (5/24). If the mother had a primary CMV infection in early pregnancy, 57% (4/7) of the children suffered from severe neurologic abnormalities and 29% (2/7) from mild neurologic abnormalities. If the mother had a non-primary infection, 25% (3/12) had severe neurologic abnormality and 25% (3/12) had mild neurologic abnormality.

Of the children with available hearing test results, SNHL was present in 42% (8/19). The average follow-up time was 44.6 months (ranging from 5 to 132 months). Of the children whose mothers had primary infection in the first trimester 20% (1/5) had bilateral and 40% (2/5) had unilateral hearing loss. Of the children whose mothers had non-primary infection, 20% (2/10) had bilateral hearing loss and 30% (3/10) had unilateral hearing loss. Three children had hearing aids, and one of them needed a cochlear implant.

The data from ophthalmological follow-up were available for 18 children with average follow-up of 21.7 months (ranging from 0 to 60 months). Two children with cerebral palsy and severe intellectual disability had visual impairment.

5.4.2 OUTOME OF INFANTS IDENTIFIED IN THE CONGENITAL CMV SCREENING (III)

5.4.2.1 Neonatal presentation

The universal screening was performed in well-baby nurseries and basic neonatal wards. All CMV-positive children were identified in the screening. None of the CMV-positive children had presented with apparent clinical symptoms raising clinical suspicion of cCMV infection. Based on clinical findings, however, 10% (4/40) of the children were categorized as having symptomatic infection. One child had microcephaly (-3.3 SD), and three children had calcifications detected in the cerebral US. Birth measures and

gestational ages at birth are presented in Table 12. One child was born prematurely at gestational week 36 +4, and all other infants were full-term.

Table 12. *Characteristics of cCMV-positive infants in the screening study.*

Characteristics	Mean	Range
Gestational age, weeks	39+4	36+4 to 42+0
Birth weight, kg	3.315	2.162 to 3.980
Birth height, cm	49.7	46 to 54
Head circumference, cm	34.5	30.0 to 36.5
Birth weight, SD[a]	-0.48	-2.1 to +1.2
Birth height, SD[a]	-0.21	-2.0 to +2.4
Head circumference, SD[a]	-0.24	-3.3 to +1.6
Apgar 1 min	8.9	6 to 10

[a]Population-based reference [259]

5.4.2.2 Imaging findings

Cranial ultrasound was performed on all 40 CMV-positive infants and 52 healthy controls at 3 months of age. Examination was not blinded. Abnormalities were seen in 11/40 CMV-positive and 3/52 healthy controls. The imaging findings of CMV-positive infants are presented in Table 13. Two healthy controls had lenticulostriatal vasculopathy, and one had small germinolytic cysts on US. MRI was performed at 6 months to 20 months of age in 5 CMV-positive children on clinical indications: three children with calcifications in US, one child with microcephaly, and one child with mildly delayed motor development.

Table 13. *Imaging findings of CMV-positive children.*

Abnormal brain ultrasound findings in 11/40 CMV positive	Abnormal cerebral MRI findings in 4/5 CMV positive
Calcifications (n=3) • small/multiple small Cysts (n=6) • periventricular • germinolytic cysts • isolated plexus cyst Lenticulostriate vasculopathy (n=4)	No findings (n=1) Increased signal intensity in white matter (n=2) Small cysts (n=2) • caudothalamic sulcus • duplex cyst in corpus pineale Calcifications (n=2) • in anterior horn unilaterally • thalamus Polymicrogyria (n=1) • in frontal lobes anterolaterally

5.4.2.3 Neurology at 18 months

The developmental outcome was evaluated at 18 months of age with Griffiths Mental Development Scales from birth to 2 years. The outcome was compared with the healthy controls. There was no difference in general quotient or quotient in any subscales at 18 months. Griffiths quotients are presented in Table 14.

Table 14. *Griffiths Developmental Scales at 18 months old did not differ between congenital CMV positive and healthy controls in any subscale or general quotient.*

Griffiths quotients	cCMV positive mean (range)	Controls mean (range)	p[a]
A Locomotor	100.8 (62–119)	100.5 (50–119)	0.715
B Personal-social	105.5 (72–124)	104.6 (63–124)	0.721
C Hearing and language	101.2 (77–127)	102.0 (79–125)	0.472
D Eye-hand coordination	103.2 (79–127)	101.7 (56–127)	0.650
E Performance	96.3 (73–123)	99.8 (50–122)	0.173
General quotient	101.0 (85–120)	101.6 (81–117)	0.557

[a]Mann-Whitney U test

5.4.2.4 Hearing at 18 months

Hearing was tested for 35 CMV-positive patients and 46 healthy controls at 18 months of age. Based on a combination of TEOAE and SF audiometry, none of the children needed hearing rehabilitation (i.e., hearing aid or cochlear implantation) by the age of 18 months. Due to insufficient cooperation or artefact noises, TEOAE was technically unsuccessful in 16/70 (23%) ears tested in CMV-positive children, and 12/92 (13%) ears tested in healthy controls. Of the technically reliable measurements, the proportion of failed TEOAEs was similar in CMV-positive (4/54) and heathy controls (6/80) (p=1.000).

5.4.2.5 Ophthalmology at 18 months

An ophthalmologist evaluated the 35 patients and 47 healthy controls at 18 months of age. No CMV-related pathologies were detected. One CMV-positive child had significant hypertrophia, a physiological finding at 3 to 18 months of age. One healthy control child had intermittent esotropia.

5.5 VIRAL SHEDDING (IV)

CMV shedding to saliva, urine, and plasma was measured at 3 months and 18 months of age in cCMV children identified in the screening. All tested urine samples were positive for CMV culture at 3 months (40/40) and 18 months (33/33) of age. Saliva samples were positive for CMV PCR in all 3-month samples (40/40) but only 24% (9/37) in 18-month samples. Plasma samples tested at 3 months of age had positive CMV PCR in nearly half (19/40) but only 6% (2/34) at 18 months of age.

5.6 DISTRIBUTION OF GENOTYPES FOR CMV ENVELOPE GLYCOPROTEINS H (*UL75*), B (*UL55*), AND N (*UL73*) (IV)

CMV-positive screening saliva samples were genotyped for CMV gH (*UL75*), gB (*UL55*), and gN (*UL73*). Follow-up saliva samples at 3 months and 18 months of age were genotyped for gH and gB. Both two genotypes for gH (gH1 and gH2) and all four genotypes for gB (gB1, gB2, gB3, and gB4) were present in our samples. All genotypes for gN except gN2 were present in our cohort (gN1, gN3a, gN3b, gN4a, gN4b, and gN4c). Mixed infections were uncommon; only two screening samples had two distinct genotypes for gH (6%) and one sample for gN (4%). Results of genotyping are presented in Table 15.

None of the genotypes for gH, gB, or gN was associated with symptomatic infection, or neurologic outcome measured with Griffiths Mental Development Scales at 18 months age.

Table 15. *Genotyping for CMV gH (genotypes gH1, gH2), gB (genotypes gB1, gB2, gB3, gB4), and gN (genotypes gN1, gN2, gN3a, gN3b, gN4a, gN4b, gN4c) in CMV-positive saliva samples in screening (gH, gB, and gN), and at 3 and 18 months old (gH and gB). Four samples had new mutations resulting in amino acid changes in gN genotype that has not been previously described (3bN1and 4aN1). NT=not tested NA=not amplified*

	Screening saliva			3-month saliva		18-month saliva		
Case	gH	gB	gN	gH	gB	CMV PCR	gH	gB
1	1	1	4c	NT	NT			
2	2	2	4b	2	2	-		
3	NT	NT	NT	2	1	-		
4	2	3	3b	2	3	+	1	4
5	1, 2	1	NT	2	1	-		
6	1	1	1	NT	NT	-		
7	1, 2	3	4c	2	3	-		
8	NT	NT	NT	NA	1	-		
9	1	1	4b	NT	NT	-		
10	1	1	1	1	1	-		
11	2	1	3b	2	1	-		
12	1	3	3bN1	NA	3	-		
13	1	2	NT	1	2	+	1	NA
14	2	2	NT	2	2	+	1	NA
15	2	1	NT	2	1	+	NA	4
16	NA	1	NT	2	1	-		
17	2	1	3b	2	1	-		
18	2	3	4c	2	3	-		
19	1	2	1	1	2	-		
20	1	3	3a	1	3	-		
21	2	1	NT	2	1	-		
22	2	1	4c	2	1	-		
23	1	2	3bN1	1	2	+	1	3
24	1	1	4c	1	1	-		
25	1	1	1	1	1	-		
26	1	1	4c	1	1	-		
27	1	1	NT	1	1	-		
28	1	1	1	1	1	-		
29	1	1	1	1	1	+	NT	NT
30	2	1	NT	2	1	+	NT	NT
31	2	1	4a	NA	1	-		
32	2	4	NT	2	4	-		
33	2	3	1, 4aN1	2	3	-		
34	2	3	3a	2	3			
35	1	2	NT	1	2	+	NT	NT
36	NA	4	NT	1,2	4	-		
37	2	3	4aN1	2	3	-		
38	NA	3	NT	2	3	+	NT	NT
39	2	2	NT	2	2			
40	NA	NA	NT	1	1	-		

6 DISCUSSION

6.1 CMV SEROPREVALENCE IN FINLAND AND PREVALENCE OF CONGENITAL CMV

6.1.1 MATERNAL SEROPREVALENCE OF CMV

In a cross-sectional study (I) from randomly selected FMC samples from 1992, 2002, and 2012, we observed a significant decrease in the CMV seroprevalence among pregnant women from 84.5% in 1992 to 71.5% in 2012. A similar decrease in CMV seroprevalence has been observed among pregnant women in Germany and Japan, and among 18- to 45-year-olds in Hong Kong [125, 262, 263].

Various factors could cause these changes. CMV infection has been shown to be more common in persons with lower socioeconomic status [55]. Well-being in Finland has improved during the recent decades. The income per household increased by over 40% from 1992 to 2012 [260]. Living conditions have also changed. In 1992, 29% of inhabitants were living in crowded situations, defined by living in apartments with less than one room per person, compared to only 17.5% in 2012 [260]. These factors most probably contribute to the decrease in seroprevalence.

Previously, two main factors have been hypothesized to have a major impact on CMV prevalence in developed countries: the frequency of breast feeding and children's attendance at daycare centers [264]. CMV is secreted to the milk of seropositive women; therefore, early infections through breast milk are common and seropositivity is thus passed naturally between generations. On the other hand, children acquiring the infection during early years of life continue to shed the infectious virus for long periods of time. In daycare centers, these infections are efficiently spread through close contact to other children and toys contaminated with CMV-positive excretions. Children attending daycare have shown to shed CMV more often than children taken care of at home [102-105]. Changes in daycare practices may influence seroprevalence observed in mothers having more than one child, and later in the next generations.

In Finland, however, the changes in breast-feeding practices obviously do not explain the observed decrease in seroprevalence. According to postnatal transmission through lactation, the feeding habits in the preceding generation reflect the possible changes in seropositivity rates. In Finland, breast feeding has been rising dramatically after its lowest years in the 1970s, when less than

10% of 6-month-old children received breast milk [265]. In 1990 and 2000, more than half of 6-month-olds were breast fed. In addition in 1995, only 26% of 3-month-old infants were exclusively breast fed [266]. In 2010, the proportion had, however, doubled to 53%. Although the length of breast feeding has increased lately, the proportion of infants receiving any breast milk has been over 90% since the 1990s [266]. The proportion of breastfed infants might be a more relevant factor influencing the seroprevalence than the length of breast feeding, since the excretion of CMV to breast milk usually takes place very early in the first weeks after delivery [143, 150, 267].

In Finland, the proportion of children attending daycare has risen in recent decades. In 1985, 44% of children aged 1 to 6 years attended daycare outside the home, in contrast to 63% in 2012 [268]. In addition, the proportion of children attending daycare in larger daycare centers instead of smaller units has risen from 56% to 76% [268]. These facts seem to suggest that the changes seen in national daycare trends do not explain the observed changes in the seroprevalence of CMV antibodies.

No major changes in the demographic characteristics of the Finnish population have been reported during the last 20 years. The proportion of children under 15 years of age was 19% in 1992 and 16% in 2012. Total fertility rate (i.e., estimated number of live-born children for one woman over her lifetime) has been similar in recent decades: 1.85 in 1992 and 1.80 in 2012 [260]. However, the proportion of immigrants has risen during the past few decades. In 2000, only 4.2% of women giving birth in Finland were born abroad. In 2012, the proportion had jumped to 9.1% [260]. One could speculate that this change would possibly increase rather than decrease the national rate of seroprevalence.

We did not evaluate the seroprevalence for CMV in the same population where the prospective newborn screening for cCMV was performed. Instead, we studied maternity cohort serum samples randomly selected from the whole of Finland. Screening for cCMV on the other hand was performed in Helsinki area hospitals (Kätilöopisto Hospital and Naistenklinikka in Helsinki, Jorvi Hospital in Espoo, and Lohja Hospital). In earlier studies from Finland, the seroprevalence among pregnant women was 70.7% in the Helsinki region and 56.3% in the Turku region. These samples were collected from 1992 to 1994 and in 2000, respectively [53, 54]. In the Helsinki area, the income of the mother was associated with seropositivity [53]. Hence, the seroprevalence ranged from 60.9% in higher-income areas to 76.4% in lower-income areas [53]. Based on these figures from earlier studies [53, 54] and our own data, we estimated that the current seroprevalence rate in the Helsinki area during screening study was between 50%–80%.

6.1.2 PREVALENCE OF CONGENITAL CMV

In the prospective screening study (III) from the saliva of infants, the prevalence of cCMV was 0.2% of all newborns. It is known that the seroprevalence of mothers is the most important factor affecting the prevalence of cCMV in offspring [70]. In our Finnish cohort of 20,000 infants, however, the prevalence was lower than we had estimated.

In other populations with around 70% seropositivity rate, the reported prevalence of cCMV infections has clearly been higher, such as 0.5% in Sweden and 0.4% in the USA [88, 91]. Similarly, in an earlier relatively small Finnish study by Granström et al, 2% (3/148) of infants were CMV-positive after birth [156]. In that study, the urine of the infants was screened after birth and three infants were excreting virus during the first days of life. The reported prevalence was ten times higher than that found in our present study. The reason for this difference is unclear, however, the small sample size (n=148) may increase the likelihood of a coincidence.

In our screening study, most of the sampling (98.3%) took place in regular wards of maternity hospitals and 1.7% in neonatal wards. Due to the low yield, the data collected from the NICU are not included in the analysis. This is important to take into account when interpreting our findings, since the prevalence of cCMV has been shown to be higher among children admitted to NICUs [70, 269, 270]. Thus, our prevalence rate of 2 in 1,000 represents the prevalence in apparently healthy infants, or infants with milder problems, who are not admitted to NICU. In a large screening study performed in 25 study sites in Japan, the prevalence for cCMV was 3.1 in 1,000 in the whole cohort [93]. The prevalence was, however, only 2.4 in 1,000 among the 14,642 children screened in the primary obstetric clinics and municipal hospitals, in contrast to 4.7 in 1,000 in the university hospitals or the governmental hospitals that care for referrals. We can only speculate whether performing the universal screening in the NICU would have affected our findings.

Raising awareness of CMV and simple hygienic precautions have shown some effect in preventing primary infections in seronegative women [239]. Revello et al found significant reduction in seroconversions among pregnant women who were instructed to wash hands frequently, not to kiss their children on the mouth, or to share utensils, food, drinks, or washcloths with the children [239]. Seroconversions occurred in only 1.2% (4/331) in the intervention group compared to 7.6% (24/315) in the group without intervention [239]. Another observational study reported lower seroconversion rates after 12 weeks gestation (0.19%) than in early pregnancy (0.42%). All seronegative women were instructed about CMV and the routes of transmission around 12 weeks of gestation, which could have resulted in lower transmission after counselling [238]. The study by Adler et al, however, showed similar

seroconversion rates (7.8%) among women receiving and not receiving information on CMV [237]. Although there is no available data on preventing non-primary CMV infections, the same measures could most likely prevent re-infections caused by new viral strains. Prevention of relapsing infections caused by reactivation of latent strains is of course impossible.

In Finland, the mothers of toddlers have been advised to prevent saliva contact with their offspring. These measures have not been taken to prevent CMV infection but to prevent transmitting *Streptococcus mutans,* the causative organism of caries. Similarly, parents have been advised not to share utensils, or food, or drinks with their toddlers. Since these measures showed to have some efficacy in one study in reducing seroconversions, they may play a role in the low prevalence of cCMV in our cohort [246].

6.1.3 FALSE POSITIVE SCREENING SAMPLES

In Study III, the proportion of false positives was high (15/55). The screening samples were regarded as false positives if the confirmatory CMV urine culture tested at 3 months was negative. The false positivity could be confirmed in 12 children with no CMV antibodies either at 3 months of age or later (Table 10). This high frequency of false positive samples is in line with the recent study by Leruez-Ville et al [85]. In their study, 41% of the screening samples were false positives. One obvious reason for this phenomenon is the PCR tests, which are too sensitive. Our findings emphasize the fact that confirmatory samples are of paramount importance whenever screening is based on PCR. One reason for these findings may also be that viral DNA contaminates the secretions in the birth canal or breast milk [101, 143-150]. In our study, four of the children who gave false positive samples (4/15, 27%) had no antibodies at 3 months of age. At this age, the antibodies measured reflect mainly maternal antibodies transferred transplacentally during the third trimester of pregnancy. This means that the proportion of seronegative mothers was the same as estimated to be in the population, indicating the source of false positive results is not necessarily maternal. Since shedding of CMV is very common, one potential source of contaminating DNA may also be the nurses who took care of the infants and sampled the salivas.

6.1.4 POSSIBLE ACQUIRED INFECTIONS

In our protocol, the control samples (urine, saliva and serum) were drawn first at 3 months of age. Some children could have acquired the CMV infection during the first 3 months of life, which may have influenced our results. Thus, it is impossible to differentiate cCMV from acquired CMV at the age of 3 months. Therefore, in future studies, the follow-up samples should be taken

earlier, preferably before the age of 3 weeks. However, in our setting, earlier sampling was not possible. The screening saliva samples were analyzed in the UAB in the USA, and due to the time required for sample logistics, the results were not available until 1-2 months of age. Therefore, the control sampling was not performed before the follow-up visit at 3 months.

We observed the same genotype (gB and gH) in both the initial sample taken at birth and later in the control sample at the age of 3 months in all 34 children with serial saliva samples genotyped. This confirms that the same viral strain was detected in screening and 3-month samples. However, it does not exclude the contamination from the maternal secretion and postnatal infection from maternal source with same strain.

6.2 OUTCOMES OF CHILDREN WITH CONGENITAL CMV

6.2.1 HEARING LOSS

To our surprise, the outcome of the CMV-positive children (n=40) in our cohort identified in the screening (III) did not differ from that of the controls at the age of 18 months. None of the 40 infected children had bilateral hearing loss and none needed hearing rehabilitation. This was an unexpected finding since in other similar screening studies bilateral hearing loss was detected in 6%–13.3% [86, 188] of the infants. The expected number of infants with bilateral hearing loss in our cohort of 40 positive infants would, according to the literature, have been 2 to 5 children. The reason for this discrepancy remains open. In our mind, the low numbers of hearing deficits in our study at the age of 18 months were not due to technical errors or low sensitivity of the hearing tests used. We used the TEOAE combined with SF audiometry at 18 months of age. These methods are known to be both sensitive and specific in detecting bilateral hearing losses requiring hearing aids. However, some cases of unilateral hearing losses may have remained unnoticed, due to insufficient cooperation with the children. TEOAE measures the responses from ears separately. However, it may be sensitive to artefact noises from the environment. If the child refuses to keep the sensors in the ears or fails to be quiet during the evaluation, the results can be unreliable. In the SF audiometry, the behavior response to sound stimuli is observed. The hearing from both ears is evaluated simultaneously, and unilateral hearing loss can therefore not be excluded by behavioral SF audiometry. On the other hand, this test gives good insight into functional hearing. In the future, later follow-up of this cohort will add information about the late-onset and progressive hearing losses in these children.

Hearing losses have been reported to occur equally often after primary and non-primary infections [198, 219]. However, the SNHL has been more often severe and bilateral after primary infections [198]. The high proportion of children infected after maternal non-primary infection (53%) in our prospective cohort may be one explanation for the favourable hearing outcome observed in our screening study.

Boppana et al observed higher proportion of premature infants (33%) among the children with cCMV and hearing loss than cCMV and normal hearing (9%)[187]. Although the screening was performed in basic neonatal wards, where most preterm infants born after 32 gestational weeks are taken care of, premature infants admitted to NICU are not presented in our screening. This may have contributed to our finding of favourable hearing outcome.

In the retrospective cohort of symptomatic cCMV children born between 2000 and 2012 (II), the hearing loss was much more frequent than among the screened children and appeared in 8/19 (42%) of children who had hearing tests results reported in the clinical files. This is in line with the literature [78, 86, 181, 186, 187, 192, 194, 197, 271, 272]. The hearing losses in our symptomatic cohort were in most cases unilateral (5/8). It should be noted, however, that the proportion of hearing loss may be an overestimation. The outcome of these patients was collected from the patient files, and in the case of some of the children (7/26), no information on any hearing evaluations was available. Furthermore, those CMV-infected children with normal hearing were more likely the ones who were not properly followed. In Finland, all children are routinely followed in child health clinics where their hearing is also regularly evaluated. Therefore, those children who developed late-onset hearing losses would most likely have been remitted to further evaluations. Thus, we believe that the children who were not followed had normal hearing.

6.2.2 OPHTHALMOLOGY

In our prospective cohort (III), no CMV-related ophthalmological findings were present. Visual impairment occurred in 2/18 (11%) of children in the retrospective cohort (II) of symptomatic infants, both severely neurologically affected children. This was consistent with previous studies since ophthalmological findings have been infrequent and mild among asymptomatic infants [208, 209].

6.2.3 NEURODEVELOPMENTAL OUTCOME

In Study III, the neurodevelopment of cCMV children (n=37) and healthy controls (n=51) was evaluated by the Griffiths Developmental Scales at 18

months of age. We could not find any differences between the infected children and the healthy controls. In previous studies, neurodevelopmental sequelae have been observed in 0%–14.2% of the evaluated children [84, 92, 181, 222, 223, 273, 274]. However, these cohorts were small (n=12–89). In a recent meta-analysis by Bartlett et al, no inferiority in the neurodevelopmental performance could, however, be observed in asymptomatic cCMV infants when compared to healthy controls [201].

The Griffiths scales evaluate five distinct areas of neurodevelopment: locomotor, personal-social, hearing and language, eye-hand coordination, and performance. Two previous studies evaluated children with cCMV and healthy controls with Griffiths scales. In a screening study from the early 1980s, Pearl et al evaluated children with cCMV and healthy controls with Griffiths scales at 2 years of age [204]. They did not find any difference when they compared the children with no neurological symptoms (n=36) and the healthy controls (n=74) [204]. Ivarsson et al, similarly, did not find a difference in Griffiths scales at 21 months of age when comparing 32 children with cCMV but without neurological symptoms or SNHL and 51 healthy controls [207]. Our analysis, however, included all infants identified in the screening, including the ones defined as symptomatic children based on either microcephaly (n=1) of calcification in the ultrasound (n=3). In Pearl's analysis, no difference was observed if the infants with neurological symptoms were excluded. They observed, however, significantly lower scores among the 5 children who had developed neurological abnormality or SNHL; not all of these symptoms were present at birth [82, 204]. In Ivarsson's study, none of the children identified in the screening had had neurological symptoms at birth. However, by the age of one year, 7/42 children had developed neurological symptoms or SNHL and they had been excluded from the analysis [207].

Our finding of favourable outcome may be linked to the fact that the children admitted to the NICU were not screened. It is possible that some sick infants with non-specific symptoms related to CMV could have been missed.

On the other hand, the neurodevelopmental outcome in our retrospective cohort of symptomatic children was no doubt abnormal: half (12/24, 50%) had clear findings. In most of those cases (7/12), the children were severely affected, whereas only 5 of them (5/12) had only minor abnormalities. These findings were in line with the previous literature [96, 199, 200].

Our findings based on these two cohorts emphasize the fact that the outcome of cCMV infection can be very variable. Symptomatic infection is a serious condition, with a very high morbidity. On the other hand, the children without any symptoms at birth seem to have a good prognosis. The infection was very rare in our population. This conclusion could be drawn both from our screening study with a low prevalence of 2 in 1,000 and also from the

retrospective study with only 29 symptomatic cCMV-infected children recognized during the 12-year period from all university hospitals in Finland. Although symptomatic CMV infection could be somewhat more frequent as we may have missed some children in the register-based retrospective cohort (II), the numbers were still very low in a country of 5.5 million inhabitants. In addition, no severely affected symptomatic children were identified during the prospective screening from September 2012 to January 2015 (III). This substantially influences the outcome results from the screening study.

6.2.4 PRIMARY AND NON-PRIMARY INFECTIONS AND CONGENITAL CMV

We were also interested in the type of maternal CMV infection, that is, whether the infection of the child was associated with a maternal primary or non-primary infection during pregnancy. The nature of the infection was based on the maternal serum sample drawn for other screening purposes during the first trimester. The definition of a non-primary infection was the presence of high avidity IgG in the early pregnancy samples. The definition of primary infection was either presence of low avidity IgG with IgM (primary infection in the first trimester) or no CMV antibodies (primary infection after the first trimester) in the early pregnancy samples. Interestingly, the proportion of non-primary infections was almost identical in both cohorts: 54% in the retrospective cohort of symptomatic infants (II) and 53% in the prospective cohort of asymptomatic infant (III).

In our retrospective cohort (II), nearly two-thirds (64%, 9/14) of children who acquired the infection after a maternal non-primary infection had long-term sequelae. Neurodevelopmental outcome was reported in 12 children infected after maternal non-primary infection and a quarter of those (25%, 3/12) had severe neurologic impairment. Mild impairment occurred in 3/12, and half of the children (6/12) infected after maternal non-primary infection had normal neurology. Long-term sequelae occurred in the majority (86%, 6/7) of the children infected after maternal primary infection in the first trimester (II). Interestingly, none of the 5 children whose mother had primary CMV infection in the second and third trimester had any sequelae. Our cohort is small. However, a recent retrospective study by Faure-Bardon et al with a large population showed similar results confirming our findings [275]. In their study, none of the 85 children who were infected after maternal primary infection in the second or third trimester had any sequelae, in contrast to 32% (35/108) after primary infection in the first trimester.

On the other hand, 6 mothers in our prospective screening cohort had experienced primary CMV infection in early pregnancy. None of the children were severely affected (III). In these cases, however, the transmission to fetus

may have occurred only later in the pregnancy. Our findings confirm the earlier understanding that both primary and non-primary infections during pregnancy may cause severe long-term sequelae [198, 200, 208, 216-219].

We believe the described studies resulted in valuable information with clinical importance. Although we found cCMV to be rare, CMV is a common virus circulating in the population. Awareness of the possible risk for the developing fetus may be a real fear for families. We hope that our studies help healthcare personnel in counselling parents about CMV infection during pregnancy. Psychological stress has been associated with higher vertical transmission rates in primary CMV infections [276]. Our results of mainly favourable outcome might lessen the inevitable stress among pregnant women suffering from CMV infection during pregnancy.

6.3 VIRAL SHEDDING AND GENOTYPES

6.3.1 GENOTYPES FOR GB, GH, AND GN AND OUTCOME OF CONGENITAL CMV

CMV is a ubiquitous virus with a wide spectrum of disease. The majority of congenitally infected infants recover without sequelae. However, some infants are severely damaged due to the infection. The factors affecting the virulence of the microbe are not clear. The wide genetic variability of certain genes makes it possible to differentiate genotypes among the CMV populations. Genes encoding for proteins involved in the immune responses, such as the envelope glycoproteins, have been hypothesized to affect the virulence [12]. In previous studies, the association of certain strains with pathogenicity and worse outcome, however, has been controversial [15, 21, 22, 25, 26, 34, 277-284]. In addition to genes encoding for envelope glycoproteins, there are numerous other genes that have an important role in helping the virus to elude immune response of the host, such as genes interfering with the function of major histocompatibility complex, genes involved in inducing apoptosis, and genes manipulating cytokine signaling [285]. In our studies, however, these other genes were not studied.

In line with other studies, our prospective study (IV) showed no association with any of the gH, gB, or gN genotypes and the symptomatic infection or severity of the disease [15, 21, 22, 34, 277-282]. Similarly, neurodevelopmental outcome measured with Griffiths Developmental Scales could not be associated with any particular genotype.

In most studies, including ours, the number of subjects has been very small. This makes it difficult to draw conclusions about the associations of individual

genotypes with clinical outcome. In a cohort of 93 cCMV infants in Italy, gN1 genotype was significantly more common among children with a good prognosis [44]. In that study, most of the children (62/93, 67%) were symptomatic. However, none of the gN genotype was significantly more common among symptomatic than asymptomatic children [44]. In a more recent study from the same author with 74 subjects, gN4 genotype was associated with symptomatic infection, and gN1 and gN3a genotypes with asymptomatic infection and favourable long-term outcome [26]. In a Spanish study of 18 symptomatic and 18 asymptomatic children with cCMV, gN1 genotype was associated with neurological findings at birth, even though it was not associated with abnormalities in imaging findings [283].

In an American cohort of 32 asymptomatic and 22 symptomatic children, the gB3 genotype was significantly more common among children with no symptoms at birth [284]. However, this can also be explained by geographical selection bias from cohorts because in that study the most asymptomatic children were recruited from Iowa and the majority of the symptomatic children from Texas, and local genotypes circulating in the various societies may be different [284]. In a Spanish cohort of 36 newborns and 10 aborted fetuses with cCMV, gB2 genotype was significantly associated with the presence of abnormal imaging findings, and gB4 was associated with better prognosis [283]. Half of the newborns in that cohort were symptomatic [283]. However, in most of the published studies, no association of any gN, gB, or gH genotype with clinical presentation or long-term outcome has been observed [15, 21, 22, 34, 277-282].

In our study, however, the infants were mostly asymptomatic, and the four infants who were categorized as symptomatic had mainly mild unapparent symptoms. Only one child had microcephalus, and three other children were categorized as symptomatic because of calcification in the brain ultrasound. All had a favourable outcome at 18 months of age. Based on this population, we cannot draw conclusions on the association of any genotype in the severe affision.

6.3.2 GENOTYPE DISTRIBUTION (GB, GH, GN)

The genotype distribution of gB, gH, and gN genotypes in our cohort of screened infants (IV) resembled that of other cohorts from other populations around the world [15, 21, 26, 27, 33-36, 277-279, 281-283, 286-288]. Most earlier studies of genotypes in cCMV populations have reported findings from clinical samples of mostly symptomatic infants or convenience samples of larger screening-based cohorts. It is interesting that in all cohorts the gH1 and gH2 genotypes were almost equally distributed, regardless of whether the cohort consisted of mainly clinical samples from symptomatic infants, or

included mainly asymptomatic children identified in screening [15, 34, 277, 279]. More variation occurred in the distribution of gB and gN genotypes [15, 21, 26, 27, 33, 35, 36, 277-279, 281-283, 286-288]. In most cohorts, including our cohort, the gB1 was most common genotype in nearly half of the cases [21, 22, 27, 277, 279, 281-284, 286, 287, 289]. The gN genotypes were distributed evenly, with no domination of any strain in any of the cohorts [15, 26, 35, 44, 277, 283]. The CMV strains identified in our cohort most likely present just the strains circulating within our society and fail to explain the low incidence of infections and disease burden in our population.

6.3.3 VIRAL SHEDDING

We also evaluated the length of viral shedding in children in the prospective cohort (IV). The shedding was clearly more persistent in urine than in saliva. At 18 months of age, all (33/33) cCMV-positive children shed virus to the urine, in contrast to only 24% (9/37) who tested positive in CMV PCR in the saliva. This is in line with previous studies that showed that secretion of the virus to urine lasted longer than to saliva [81, 223, 290]. Forner et al also studied the kinetics of CMV DNAemia in serial blood samples of cCMV infants [223]. Their finding was similar to our cohort: half of the children had CMV DNA detected in the blood at 3 months of age.

Salivary shedding among toddlers is an important contributor to horizontal transmission of the virus. When prevention strategies are considered among infants and toddlers, it is clearly more difficult to prevent contact with saliva and oral secretions than urine. It is much easier to recommend hand washing after every diaper change than urge hand washing after every sneeze, cough, or handling with saliva-contaminated toys. Hand washing is effective in preventing transmission from hands after contact with CMV-positive secretions [291]. In a study evaluating the survival of CMV, viable virus could be recovered from unwashed hands in almost all (18/20) hands one minute after the inoculation. At 15 minutes after inoculation, the virus could still be cultured from nearly a quarter (4/20) of tested hands. However, washing hands with either plain soap, antibacterial soap containing benzalkonium chloride, or water only eliminated viable viruses from all tested hands [291]. The CMV is not a very contagious virus, and transmission through respiratory droplets is unlikely. Direct contact with secretions containing the virus is needed for transmission. Toddlers excreting the virus to saliva may contaminate the environment more efficiently compared to children excreting the virus to urine.

The detection of CMV viruses in the saliva of healthy children with no clinical symptoms of CMV infection is common [102, 103, 105, 292]. In a French study, a fifth (80/369, 21.7%) of young children attending the emergency unit and

half (133/256, 51.9%) of children attending daycare centers were asymptomatic carriers and shed CMV in saliva [105]. Other similar studies executed in daycare center surroundings have shown CMV excretion to saliva in 45% (13/29), 10% (4/39), 10% (4/41), and 22% (12/54) of healthy children, respectively [102, 103, 292]. In our study, 24% of cCMV children tested saliva-positive at 18 months of age, which is of the same magnitude as reported in the literature among healthy children. This emphasizes the fact, that in healthcare settings, the cCMV infants should be treated with standard hygienic precautions, as any other children. In conclusion, to prevent occupational transmission of CMV from infants and children to daycare or healthcare personnel, all children should be treated as potential sources of infectious agents. Thus, it is important to follow universal precautions at all times regardless of the CMV status of the child or patient.

6.4 PUBLIC HEALTH SIGNIFICANCE

The burden of cCMV in Finland was low. Based on our findings and previous literature, approximately 11 to 15 children with permanent long-term sequelae due to the cCMV infection are born in Finland annually (Figure 8).

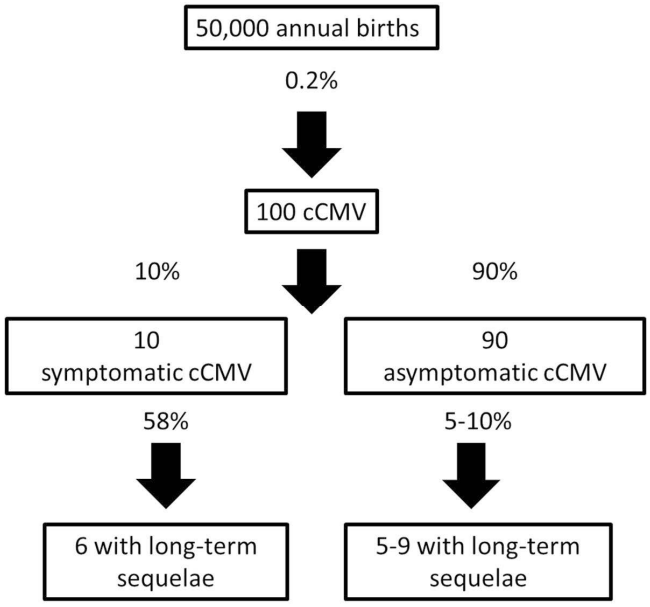

Figure 8 An estimated disease burden of congenital CMV infection in Finland, based on literature and our results in Studies II-III. The prevalence of congenital CMV was 0.2% in the prospective Study III and 10% of infected infants were symptomatic. Long-term sequelae appeared in 58% of symptomatic infants in the retrospective cohort in Study II. In the literature, it is estimated that 5%-10% of asymptomatic infants will have long-term sequelae, not confirmed by our 18-month follow-up in Study III.

6.5 ETHICAL CONSIDERATIONS

Screening of apparently well children for a potentially severe condition has ethical considerations, especially in the case of a condition with no effective intervention to prevent long-term sequelae, as is the case with cCMV. One major issue is the anxiety the family of a CMV-positive infant may experience. Our response to this potential concern was to give the families access to the appropriate and correct information. We did our best to inform the families as appropriately as possible from the beginning of the study. The families got both written information and contact information. A trained pediatrician (LP) met all the families and was available for questions by phone before the appointment. In addition, the cCMV-positive children were followed up very closely and regularly. The rehabilitative interventions, such as hearing, visual rehabilitation, or physiotherapy, were available for any child who needed them.

6.6 STRENGTHS AND LIMITATIONS

Our study provides comprehensive insight into the current situation of cCMV in Finland. The population-based screening of 19,868 infants and the prospective follow-up of 40 infected infants and 54 healthy controls comprised a unique cohort of an unselected population (III). All children born in the Helsinki area were eligible for screening. The adherence to follow-up was good. The neurologic follow-up was completed by the majority of the CMV-positive (37/40, 92.5%) and healthy controls (51/54, 94.4%). The audiologic follow-up was completed by most of the CMV-positive (35/40, 87.5%) and healthy controls (46/54, 85.2%). Similarly, the ophthalmologic follow-up was completed by most of the CMV-positive (35/40, 87.5%) and healthy controls (47/54, 87.9%).

The prevalence of cCMV was only 0.2%. When the study was designed in 2011, we estimated the prevalence to be higher. Based on the literature on other populations with similar seroprevalence of CMV, we calculated the prevalence to be 0.4%–0.5% in the Finnish population [88, 173]. If that would have been the case, our study would have identified 80–100 cCMV-positive infants instead of the 40 children diagnosed.

The same clinician (LP) carried out the pediatric examination including the Griffiths Developmental evaluation of all children who participated in the prospective study. These evaluations were, however, not blinded. In our opinion, such a blinding would have been unethical. We felt that it was crucial for the families of cCMV-positive children to meet a pediatrician at every visit

who was ready to face their questions regarding the infection and the whole study. Blinding would have made such consultations impossible.

The control samples from screening-positive infants were collected at 3 months of age. After perinatal and postnatal infections, viral shedding begins after 3 weeks of incubation. Thus, in our study we could not definitely exclude that some children in our study would have acquired the infection postnatally. As the percentage of false positive screening samples was high (15/55), earlier sampling would have been better.

In the retrospective study of symptomatic infants, the outcome data were collected from clinical files of different hospitals (II). This source of information contains risk for bias. Missing and inaccurate data were common, and the location and identification of patients were incomplete; in other words, we certainly missed some patients.

We evaluated the seroprevalence for CMV in 600 pregnant women at three time points: 1992, 2002, and 2012. The sample size of each cohort was only 200 per year. Although the samples were selected randomly from the FMC, it is possible that some selection bias in the population at different time points could have an effect on the outcome. However, since we observed a very linear decrease in seroprevalence from 1992 (84.5%) to 2002 (77.5%) and to 2012 (71.5%), we believe the data reliably represent a trend of decreasing seroprevalence for CMV in our community.

6.7 FUTURE CONSIDERATIONS

It should be recognized that examining higher cognitive abilities at the age of 18 months is challenging and that further studies performed with older children are more informative. A later follow-up of the cCMV cohort identified in the screening will give more information on the long-term cognitive outcome and identify the children with later-onset hearing losses.

In our studies the CMV-positive samples were evaluated for only three genes encoding for envelope glycoproteins of the virus. The new next-generations sequencing methods, however, would give much more data on the sequence variation among different CMV populations [12, 293]. It could be interesting to compare the CMV strains identified in our screening to other CMV populations with these sensitive technologies.

6.8 CONCLUSIONS

1. Seroprevalence for CMV among pregnant women in Finland decreased from 84.5% in 1992 to 71.5% in 2012. (I)

2. The prevalence of the cCMV infection was only 2 out of 1,000. The outcome of infected infants identified in the screening did not differ from the healthy controls at 18 months of age. (III)

3. The outcome of the 26 children with symptomatic cCMV diagnosed during the 12-year study period covering all national university hospitals was poor; 58% of them had long-term sequelae in later follow-up. The main problems were SNHL and neurological impairment. (II)

4. Maternal CMV infections were non-primary in more than half of the cases in both asymptomatic and symptomatic cCMV infections. Long-term sequelae among cCMV-positive nfants occurred after both primary and non-primary maternal infections. (II, III)

5. In Finland, the viral genotype distribution for genes encoding for viral envelope glycoproteins gH, gB, and gN was similar compared to other cohorts in the literature. Thus, the discovered genotypes do not explain the low burden of cCMV in our population. CMV shedding in urine was more prolonged than to saliva among congenitally infected children. (IV)

In summary, the disease burden of cCMV in Finland was lower than we estimated. The low prevalence of cCMV and the good clinical outcome of the asymptomatic infants suggests to us that universal screening of Finnish children seems unwarranted at the moment.

7 ACKNOWLEDGEMENTS

This study was carried out from 2012–2019 at the Children's Hospital, Pediatric Research Center, University of Helsinki and Helsinki University hospital. I owe my greatest gratitude to everyone who has contributed to the completion of this thesis. I am most grateful to professor Markku Heikinheimo, professor Sture Andersson, and professor Kaija-Leena Kolho of the Children's Hospital, University of Helsinki, and Helsinki University Hospital. I wish to thank professor Kim Vettenranta, docent Turkka Kirjavainen, and docent Jussi Merenmies, the present and former programme directors of the doctoral programme at the Childrens's Hospital, and professor Taneli Raivio, head of Pediatric Research Center, for providing excellent facilities for research. I owe gratitude to Jari Petäjä, Director of Children's Hospital; Anne Wikström, Head of Pediatrics; Eero Jokinen, Head of Tertiary Pediatrics; and Marjo Metsäranta, head of Neonatology.

The study was supported by the Finnish Government Research funding, Päivikki and Sakari Sohlberg Foundation, Yrjö Jahnsson Foundation, Pediatric Research Foundation, Arvo and Lea Ylppö Foundation, Finnish Medical Foundation, Hearing Foundation, Finnish Brain Foundation, Alfred Kordelin Foundation, and Lastenlinna Foundation. I am extremely grateful for their support, since the studies could not have been carried out without the financial facilities provided by the funders.

I owe my greatest gratitude for all the children and families who participated in the studies. I sincerely hope the studies will help children and families with cCMV infection in the future.

My warmest gratitude goes to my supervisor, professor Harri Saxen, for his encouragement and support during this project. I really have been privileged in having you as my supervisor. Your huge scientific and clinical expertise combined with human way of thinking has created a supportive working atmosphere. You have always been there for any questions. During this project, I never ceased to be surprised by how quickly you replied to my emails, no matter whether you were on holiday or in the office. If I had any doubts for the project, the conversations with you always succeeded in raising my enthusiasm and belief in this project again.

I am grateful to our superb study group for the CMV screening study. I wish to thank Tuula Lönnqvist, Maija Lappalainen, Riina Niemensivu, Päivi Lindahl, Irmeli Nupponen, Tea Nieminen, and Raija Seuri for your invaluable ideas in designing the study and your support during all the study years. I am extremely grateful to our collaborators in University of Alabama, Birmingham.

I want to thank Suresh Boppana for sharing us with your exceptional expertise on cCMV. I am grateful for all the help received from Sunil Pati and Nazma Chowdhury during the project. I want to thank the collaborators in our retrospective study, Merja Helminen, Marjo Renko, Ville Peltola, and Tarja Heiskanen-Kosma. Your help was essential in the data collection. I am grateful for Heljä-Marja Surcel for the essential help in providing the serum bank sera for the studies. Thank you, Emmi Sarvikivi, for your help in statistics.

I am deeply grateful for our study nurse, Satu Lindström, for the invaluable help throughout all the years on this project. Not only all the practical things and organizing that made this project possible, but also the priceless friendship. We have laughed a lot and also cried a bit. Working with you has made this project a pleasure.

I wish to thank audiologist, Tiina Kuntonen, and ophthalmologist, Suvi Viskari, for the contributions to the study. I am most grateful to all the nurses in the well-baby nurseries and neonatal wards for their substantial work in sampling almost 20,000 saliva samples.

I am grateful to the members of the thesis committee, docent Irmeli Lautenschlager and docent Martin Renlund for your interest in our studies and your advice and time evaluating the project. I warmly thank the official reviewers, docent Kaarin Mäkikallio and docent Veijo Hukkanen, for your excellent comments and constructive criticism on the thesis.

I have been extremely happy to get to know people around the world in the context of cCMV. I want to thank Marjolein Korndewal, Fleurtje Schornagel, Ann Vossen, Roberta Rovito, Julia Gunkel, Horst Buxmann, Sue Luck, Shannon Ross, Karen Fowler, Eva Karltorp, Ulrika Löfkvist, Marianne Forsgren, Regine Barlinn, and others for the interesting and educational conversations about cCMV infection.

I want to thank all the co-workers on the Biomedicum sixth floor. The support in all minor and major practical things has been invaluable. Thanks to Susann, Sonja, Antti, Laura K, Silja, Mari, Johanna, Matti, Juuso, Helena, Elisa, Jesper, Tero, Satu P, Anu S, Maarit, Päivi, Anne, Rhea, and Marjaana. Thank you, Jenni, for the extremely valuable peer support in many things, including thesis writing. I sincerely thank the other pediatric infectious diseases colleagues Satu K, Verna, Eeva, Tuula, Svetlana, Otto, and all other colleagues in New Children's Hospital and Jorvi.

I am grateful to all my friends. Thank you, Johanna, Mikko, Paula, Terhi, Mats, Anna, Katri, Krista, Heini, and Markku. Thank you, Omar for fixing the Griffiths equipment, and thank you, Esko for providing transport always

wherever needed, including transporting children to and from their hobbies when we were unable.

I owe my greatest gratitude to my parents for all the unconditional love and care. My mother, you taught me to work hard for my dreams. Your memory will live forever. My father, you have always been there for me and my family. Thank you. I am deeply grateful to my brother Tarmo and Minh and your lovely daughter Emma. I want to thank my parents-in-law, Tuula and Pertti, my sister-in-law Heli and Saku, and Emma and Eero. I want to thank all my dear relatives for just being there. Thank you for your support, love and faith in me.

Finally, I owe my deepest gratitude to my family. My dear husband Olli, I thank you for your support and belief in me. I admire your ability to take on new challenges and fulfilling your dreams. I want to thank my precious children, Veera and Topias. Thank you for pushing me to get this done. Thank you and sorry! I promise to be more present in the future. I promise!

8 REFERENCES

1. Ribbert H. Uber protozoenartige zellen in der niere eines syphilitischen neugeborenen und in der parotis von kindern. Zentralbl Allg Pathol, 1904; 15: 945.

2. Ho M. The history of cytomegalovirus and its diseases. Med Microbiol Immunol, 2008; 197: 65-73.

3. Jesionek A, Kiolemenoglou B. Uber einen befund von protozoenartigen gebilden in den organen eines hereditar-luetischen foetus. Muenchener Med Wochenschr, 1904; 51: 1905.

4. Wyatt JP, Saxton J. Generalized cytomegalic inclusion disease. J Pediatr, 1950; 36: 271-94, illust.

5. Riley HD, Jr. History of the cytomegalovirus. South Med J, 1997; 90: 184-90.

6. Smith MG. Propagation in tissue cultures of a cytopathogenic virus from human salivary gland virus (SGV) disease. Proc Soc Exp Biol Med, 1956; 92: 424-30.

7. Rowe WP, Hartley JW, Waterman S, Turner HC, Huebner RJ. Cytopathogenic agent resembling human salivary gland virus recovered from tissue cultures of human adenoids. Proc Soc Exp Biol Med, 1956; 92: 418-24.

8. Craig JM, Macauley JC, Weller TH, Wirth P. Isolation of intranuclear inclusion producing agents from infants with illnesses resembling cytomegalic inclusion disease. Proc Soc Exp Biol Med, 1957; 94: 4-12.

9. Klemola E, Kaariainen L. Cytomegalovirus as a possible cause of a disease resembling infectious mononucleosis. Br Med J, 1965; 2: 1099-102.

10. Yu X, Jih J, Jiang J, Zhou ZH. Atomic structure of the human cytomegalovirus capsid with its securing tegument layer of pp150. Science, 2017; 356: 10.1126/science.aam6892.

11. Kalejta RF. Tegument proteins of human cytomegalovirus. Microbiol Mol Biol Rev, 2008; 72: 249-65.

12. Arav-Boger R. Strain variation and disease severity in congenital cytomegalovirus infection: In search of a viral marker. Infect Dis Clin North Am, 2015; 29: 401-14.

13. Gibson W. Human cytomegalovirus: Molecular biology. In: Mahy BWJ, Van Regenmortel MHV. Encyclopedia of Virology. Third Edition ed. Academic Press, 2008: 485-90.

14. Mocarski ESJ, Pass RF. Human cytomegalovirus: General features. In: Mahy BWJ, Van Regenmortel MHV. Encyclopedia of Virology. Third Edition ed. Academic Press, 2008: 474-85.

15. Pati SK, Pinninti S, Novak Z, et al. Genotypic diversity and mixed infection in newborn disease and hearing loss in congenital cytomegalovirus infection. Pediatr Infect Dis J, 2013; 32: 1050-4.

16. Arav-Boger R, Willoughby RE, Pass RF, et al. Polymorphisms of the cytomegalovirus (CMV)-encoded tumor necrosis factor-alpha and beta-chemokine receptors in congenital CMV disease. J Infect Dis, 2002; 186: 1057-64.

17. Arav-Boger R, Foster CB, Zong JC, Pass RF. Human cytomegalovirus-encoded alpha -chemokines exhibit high sequence variability in congenitally infected newborns. J Infect Dis, 2006; 193: 788-91.

18. Arav-Boger R, Battaglia CA, Lazzarotto T, et al. Cytomegalovirus (CMV)-encoded UL144 (truncated tumor necrosis factor receptor) and outcome of congenital CMV infection. J Infect Dis, 2006; 194: 464-73.

19. Waters A, Hassan J, De Gascun C, et al. Human cytomegalovirus UL144 is associated with viremia and infant development sequelae in congenital infection. J Clin Microbiol, 2010; 48: 3956-62.

20. Picone O, Costa JM, Chaix ML, Ville Y, Rouzioux C, Leruez-Ville M. Human cytomegalovirus UL144 gene polymorphisms in congenital infections. J Clin Microbiol, 2005; 43: 25-9.

21. Nijman J, Mandemaker FS, Verboon-Maciolek MA, et al. Genotype distribution, viral load and clinical characteristics of infants with postnatal or congenital cytomegalovirus infection. PLoS One, 2014; 9: e108018.

22. Yan H, Koyano S, Inami Y, et al. Genetic variations in the gB, UL144 and UL149 genes of human cytomegalovirus strains collected from congenitally and postnatally infected japanese children. Arch Virol, 2008; 153: 667-74.

23. Heo J, Petheram S, Demmler G, et al. Polymorphisms within human cytomegalovirus chemokine (UL146/UL147) and cytokine receptor genes (UL144) are not predictive of sequelae in congenitally infected children. Virology, 2008; 378: 86-96.

24. Paradowska E, Jablonska A, Plociennikowska A, et al. Cytomegalovirus alpha-chemokine genotypes are associated with clinical manifestations in

children with congenital or postnatal infections. Virology, 2014; 462-463: 207-17.

25. Pignatelli S, Dal Monte P, Rossini G, Lazzarotto T, Gatto MR, Landini MP. Intrauterine cytomegalovirus infection and glycoprotein N (gN) genotypes. J Clin Virol, 2003; 28: 38-43.

26. Pignatelli S, Lazzarotto T, Gatto MR, et al. Cytomegalovirus gN genotypes distribution among congenitally infected newborns and their relationship with symptoms at birth and sequelae. Clin Infect Dis, 2010; 51: 33-41.

27. Yamamoto AY, Mussi-Pinhata MM, de Deus Wagatsuma VM, Marin LJ, Duarte G, Figueiredo LT. Human cytomegalovirus glycoprotein B genotypes in brazilian mothers and their congenitally infected infants. J Med Virol, 2007; 79: 1164-8.

28. Stanton R, Westmoreland D, Fox JD, Davison AJ, Wilkinson GW. Stability of human cytomegalovirus genotypes in persistently infected renal transplant recipients. J Med Virol, 2005; 75: 42-6.

29. Baldanti F, Sarasini A, Furione M, et al. Coinfection of the immunocompromised but not the immunocompetent host by multiple human cytomegalovirus strains. Arch Virol, 1998; 143: 1701-9.

30. Puchhammer-Stockl E, Gorzer I, Zoufaly A, et al. Emergence of multiple cytomegalovirus strains in blood and lung of lung transplant recipients. Transplantation, 2006; 81: 187-94.

31. Chou SW. Acquisition of donor strains of cytomegalovirus by renal-transplant recipients. N Engl J Med, 1986; 314: 1418-23.

32. Ross SA, Novak Z, Pati S, et al. Mixed infection and strain diversity in congenital cytomegalovirus infection. J Infect Dis, 2011; 204: 1003-7.

33. Rycel M, Wujcicka W, Zawilinska B, et al. Mixed infections with distinct cytomegalovirus glycoprotein B genotypes in polish pregnant women, fetuses, and newborns. Eur J Clin Microbiol Infect Dis, 2015; 34: 585-91.

34. Paradowska E, Jablonska A, Studzinska M, et al. Cytomegalovirus glycoprotein H genotype distribution and the relationship with hearing loss in children. J Med Virol, 2014; 86: 1421-7.

35. Garcia de Figueiredo G, Marques AA, Mussi-Pinhata MM, Silva WA, Jr, Yamamoto AY. Is the mixture of human cytomegalovirus genotypes frequent in infants with congenital infection at birth in a high seroprevalence population? J Med Virol, 2018; 90: 1389-97.

36. Correa C, Kouri V, Perez L, Soto Y, Limia C. Diagnosis, gB genotype distribution and viral load of symptomatic congenitally infected CMV patients in cuba. J Perinatol, 2016; 36: 837-42.

37. Isaacson MK, Compton T. Human cytomegalovirus glycoprotein B is required for virus entry and cell-to-cell spread but not for virion attachment, assembly, or egress. J Virol, 2009; 83: 3891-903.

38. Kinzler ER, Theiler RN, Compton T. Expression and reconstitution of the gH/gL/gO complex of human cytomegalovirus. J Clin Virol, 2002; 25 Suppl 2: S87-95.

39. Kinzler ER, Compton T. Characterization of human cytomegalovirus glycoprotein-induced cell-cell fusion. J Virol, 2005; 79: 7827-37.

40. Dal Monte P, Pignatelli S, Mach M, Landini MP. The product of human cytomegalovirus UL73 is a new polymorphic structural glycoprotein (gpUL73). J Hum Virol, 2001; 4: 26-34.

41. Pignatelli S, Dal Monte P, Landini MP. gpUL73 (gN) genomic variants of human cytomegalovirus isolates are clustered into four distinct genotypes. J Gen Virol, 2001; 82: 2777-84.

42. Mach M, Kropff B, Kryzaniak M, Britt W. Complex formation by glycoproteins M and N of human cytomegalovirus: Structural and functional aspects. J Virol, 2005; 79: 2160-70.

43. Kropff B, Burkhardt C, Schott J, et al. Glycoprotein N of human cytomegalovirus protects the virus from neutralizing antibodies. PLoS Pathog, 2012; 8: e1002999.

44. Pignatelli S, Dal Monte P, Rossini G, et al. Human cytomegalovirus glycoprotein N (gpUL73-gN) genomic variants: Identification of a novel subgroup, geographical distribution and evidence of positive selective pressure. J Gen Virol, 2003; 84: 647-55.

45. Bates M, Monze M, Bima H, et al. High human cytomegalovirus loads and diverse linked variable genotypes in both HIV-1 infected and exposed, but uninfected, children in Africa. Virology, 2008; 382: 28-36.

46. Plotkin SA, Boppana SB. Vaccination against the human cytomegalovirus. Vaccine, 2018. Epub ahead of print.

47. Antona D, Lepoutre A, Fonteneau L, et al. Seroprevalence of cytomegalovirus infection in France in 2010. Epidemiol Infect, 2017; 145: 1471-8.

48. Hassan J, O'Neill D, Honari B, et al. Cytomegalovirus infection in Ireland: Seroprevalence, HLA class I alleles, and implications. Medicine (Baltimore), 2016; 95: e2735.

49. Korndewal MJ, Mollema L, Tcherniaeva I, et al. Cytomegalovirus infection in the Netherlands: Seroprevalence, risk factors, and implications. J Clin Virol, 2015; 63: 53-8.

50. Lanzieri TM, Dollard SC, Bialek SR, Grosse SD. Systematic review of the birth prevalence of congenital cytomegalovirus infection in developing countries. Int J Infect Dis, 2014; 22: 44-8.

51. Wang S, Wang T, Zhang W, et al. Cohort study on maternal cytomegalovirus seroprevalence and prevalence and clinical manifestations of congenital infection in china. Medicine (Baltimore), 2017; 96: e6007.

52. Mussi-Pinhata MM, Yamamoto AY, Aragon DC, et al. Seroconversion for cytomegalovirus infection during pregnancy and fetal infection in a highly seropositive population: "The BraCHS study". J Infect Dis, 2018; 218: 1200-4.

53. Mustakangas P, Sarna S, Ammala P, Muttilainen M, Koskela P, Koskiniemi M. Human cytomegalovirus seroprevalence in three socioeconomically different urban areas during the first trimester: A population-based cohort study. Int J Epidemiol, 2000; 29: 587-91.

54. Alanen A, Kahala K, Vahlberg T, Koskela P, Vainionpaa R. Seroprevalence, incidence of prenatal infections and reliability of maternal history of varicella zoster virus, cytomegalovirus, herpes simplex virus and parvovirus B19 infection in south-western finland. BJOG, 2005; 112: 50-6.

55. Cannon MJ, Schmid DS, Hyde TB. Review of cytomegalovirus seroprevalence and demographic characteristics associated with infection. Rev Med Virol, 2010; 20: 202-13.

56. Lantos PM, Hoffman K, Permar SR, et al. Neighborhood disadvantage is associated with high cytomegalovirus seroprevalence in pregnancy. J Racial Ethn Health Disparities, 2018; 5: 782-6.

57. Lantos PM, Hoffman K, Permar SR, Jackson P, Hughes BL, Swamy GK. Geographic disparities in cytomegalovirus infection during pregnancy. J Pediatric Infect Dis Soc, 2017; 6: e55-61.

58. Barlinn R, Dudman SG, Trogstad L, et al. Maternal and congenital cytomegalovirus infections in a population-based pregnancy cohort study. APMIS, 2018; 126: 899-906.

59. Engman ML, Malm G, Engstrom L, et al. Congenital CMV infection: Prevalence in newborns and the impact on hearing deficit. Scand J Infect Dis, 2008; 40: 935-42.

60. Pembrey L, Raynor P, Griffiths P, Chaytor S, Wright J, Hall AJ. Seroprevalence of cytomegalovirus, Epstein Barr virus and varicella zoster virus among pregnant women in bradford: A cohort study. PLoS One, 2013; 8: e81881.

61. Lachmann R, Loenenbach A, Waterboer T, et al. Cytomegalovirus (CMV) seroprevalence in the adult population of Germany. PLoS One, 2018; 13: e0200267.

62. Barbi M, Binda S, Caroppo S, et al. Multicity Italian study of congenital cytomegalovirus infection. Pediatr Infect Dis J, 2006; 25: 156-9.

63. Lopo S, Vinagre E, Palminha P, Paixao MT, Nogueira P, Freitas MG. Seroprevalence to cytomegalovirus in the Portuguese population, 2002-2003. Euro Surveill, 2011; 16: 19896.

64. Kuessel L, Husslein H, Marschalek J, et al. Prediction of maternal cytomegalovirus serostatus in early pregnancy: A retrospective analysis in Western Europe. PLoS One, 2015; 10: e0145470.

65. Lanzieri TM, Kruszon-Moran D, Gambhir M, Bialek SR. Influence of parity and sexual history on cytomegalovirus seroprevalence among women aged 20-49 years in the USA. Int J Gynaecol Obstet, 2016; 135: 82-5.

66. Shigemi D, Yamaguchi S, Otsuka T, Kamoi S, Takeshita T. Seroprevalence of cytomegalovirus IgG antibodies among pregnant women in Japan from 2009-2014. Am J Infect Control, 2015; 43: 1218-21.

67. Shaiegan M, Rasouli M, Zadsar M, Zolfaghari S. Meta-analysis of cytomegalovirus seroprevalence in volunteer blood donors and healthy subjects in iran from 1992 to 2013. Iran J Basic Med Sci, 2015; 18: 627-34.

68. El Sanousi SM, Osman ZA, Mohmed AB, Al Awfi MS. Cytomegalovirus infection in a cohort of pregnant women. Am J Infect Control, 2016; 44: e41-3.

69. Alvarado-Esquivel C, Terrones-Saldivar MDC, Hernandez-Tinoco J, et al. Seroepidemiology of cytomegalovirus infection in pregnant women in the central Mexican city of Aguascalientes. J Clin Med Res, 2018; 10: 337-44.

70. Kenneson A, Cannon MJ. Review and meta-analysis of the epidemiology of congenital cytomegalovirus (CMV) infection. Rev Med Virol, 2007; 17: 253-76.

71. Dollard SC, Grosse SD, Ross DS. New estimates of the prevalence of neurological and sensory sequelae and mortality associated with congenital cytomegalovirus infection. Rev Med Virol, 2007; 17: 355-63.

72. Wang C, Zhang X, Bialek S, Cannon MJ. Attribution of congenital cytomegalovirus infection to primary versus non-primary maternal infection. Clin Infect Dis, 2011; 52: e11-3.

73. de Vries JJ, van Zwet EW, Dekker FW, Kroes AC, Verkerk PH, Vossen AC. The apparent paradox of maternal seropositivity as a risk factor for congenital cytomegalovirus infection: A population-based prediction model. Rev Med Virol, 2013; 23: 241-9.

74. Cutts FT, Vynnycky E. Modelling the incidence of congenital rubella syndrome in developing countries. Int J Epidemiol, 1999; 28: 1176-84.

75. Netto EM, Moreira-Soto A, Pedroso C, et al. High Zika virus seroprevalence in Salvador, Northeastern Brazil limits the potential for further outbreaks. MBio, 2017; 8: 10.1128/mBio.01390-17.

76. Paixao P, Almeida S, Gouveia P, Vilarinho L, Vaz Osorio R. Prevalence of human cytomegalovirus congenital infection in Portuguese newborns. Euro Surveill, 2009; 14: 13-5.

77. Marin LJ, Santos de Carvalho Cardoso E, Bispo Sousa SM, et al. Prevalence and clinical aspects of CMV congenital infection in a low-income population. Virol J, 2016; 13: 148.

78. Yamamoto AY, Mussi-Pinhata MM, Isaac Mde L, et al. Congenital cytomegalovirus infection as a cause of sensorineural hearing loss in a highly immune population. Pediatr Infect Dis J, 2011; 30: 1043-6.

79. Zhang XW, Li F, Yu XW, Shi XW, Shi J, Zhang JP. Physical and intellectual development in children with asymptomatic congenital cytomegalovirus infection: A longitudinal cohort study in Qinba mountain area, China. J Clin Virol, 2007; 40: 180-5.

80. van der Sande MA, Kaye S, Miles DJ, et al. Risk factors for and clinical outcome of congenital cytomegalovirus infection in a peri-urban West-African birth cohort. PLoS One, 2007; 2: e492.

81. MacDonald H, Tobin JO. Congenital cytomegalovirus infection: A collaborative study on epidemiological, clinical and laboratory findings. Dev Med Child Neurol, 1978; 20: 471-82.

82. Peckham CS, Chin KS, Coleman JC, Henderson K, Hurley R, Preece PM. Cytomegalovirus infection in pregnancy: Preliminary findings from a prospective study. Lancet, 1983; 1: 1352-5.

83. Waters A, Jennings K, Fitzpatrick E, et al. Incidence of congenital cytomegalovirus infection in Ireland: Implications for screening and diagnosis. J Clin Virol, 2014; 59: 156-60.

84. Barbi M, Binda S, Primache V, Clerici D. Congenital cytomegalovirus infection in a northern Italian region. NEOCMV group. Eur J Epidemiol, 1998; 14: 791-6.

85. Leruez-Ville M, Magny JF, Couderc S, et al. Risks factors for congenital CMV infection following primary and non-primary maternal infection: A prospective neonatal screening study using PCR in saliva. Clin Infect Dis, 2017; 65: 398-404.

86. Foulon I, Naessens A, Foulon W, Casteels A, Gordts F. A 10-year prospective study of sensorineural hearing loss in children with congenital cytomegalovirus infection. J Pediatr, 2008; 153: 84-8.

87. de Vries JJ, Korver AM, Verkerk PH, et al. Congenital cytomegalovirus infection in the Netherlands: Birth prevalence and risk factors. J Med Virol, 2011; 83: 1777-82.

88. Ahlfors K, Ivarsson SA, Harris S. Report on a long-term study of maternal and congenital cytomegalovirus infection in Sweden. Review of prospective studies available in the literature. Scand J Infect Dis, 1999; 31: 443-57.

89. Paradiz KR, Seme K, Puklavec E, Paro-Panjan D, Poljak M. Prevalence of congenital cytomegalovirus infection in Slovenia: A study on 2,841 newborns. J Med Virol, 2012; 84: 109-15.

90. Barkai G, Ari-Even Roth D, Barzilai A, et al. Universal neonatal cytomegalovirus screening using saliva - report of clinical experience. J Clin Virol, 2014; 60: 361-6.

91. Ross SA, Ahmed A, Palmer AL, et al. Newborn dried blood spot polymerase chain reaction to identify infants with congenital cytomegalovirus-associated sensorineural hearing loss. J Pediatr, 2017; 184: 57-61.e1.

92. Numazaki K, Fujikawa T. Chronological changes of incidence and prognosis of children with asymptomatic congenital cytomegalovirus infection in Sapporo, Japan. BMC Infect Dis, 2004; 4: 22.

93. Koyano S, Inoue N, Oka A, et al. Screening for congenital cytomegalovirus infection using newborn urine samples collected on filter paper: Feasibility and outcomes from a multicentre study. BMJ Open, 2011; 1: e000118.

94. Yamagishi Y, Miyagawa H, Wada K, et al. CMV DNA detection in dried blood spots for diagnosing congenital CMV infection in Japan. J Med Virol, 2006; 78: 923-5.

95. Yamaguchi A, Oh-Ishi T, Arai T, et al. Screening for seemingly healthy newborns with congenital cytomegalovirus infection by quantitative real-time polymerase chain reaction using newborn urine: An observational study. BMJ Open, 2017; 7: e013810.

96. Nishida K, Morioka I, Nakamachi Y, et al. Neurological outcomes in symptomatic congenital cytomegalovirus-infected infants after introduction of newborn urine screening and antiviral treatment. Brain Dev, 2015; 38: 209-16.

97. Tanimura K, Tairaku S, Morioka I, et al. Universal screening with use of immunoglobulin G avidity for congenital cytomegalovirus infection. Clin Infect Dis, 2017; 65: 1652-8.

98. Marin LJ, Santos de Carvalho Cardoso E, Bispo Sousa SM, et al. Prevalence and clinical aspects of CMV congenital infection in a low-income population. Virol J, 2016; 13: 148.

99. Karimian P, Yaghini O, Nasr Azadani H, et al. Prevalence, characteristics, and one-year follow-up of congenital cytomegalovirus infection in Isfahan City, Iran. Interdiscip Perspect Infect Dis, 2016; 7812106.

100. Viswanathan R, Bafna S, Mergu R, et al. Direct saliva real time polymerase chain reaction assay shows low birth prevalence of congenital CMV infection in urban Western India. Pediatr Infect Dis J, 2018; 38: e65-68.

101. Cannon MJ, Hyde TB, Schmid DS. Review of cytomegalovirus shedding in bodily fluids and relevance to congenital cytomegalovirus infection. Rev Med Virol, 2011; 21: 240-55.

102. Pass RF, August AM, Dworsky M, Reynolds DW. Cytomegalovirus infection in day-care center. N Engl J Med, 1982; 307: 477-9.

103. Kashiwagi Y, Nemoto S, Hisashi, et al. Cytomegalovirus DNA among children attending two day-care centers in Tokyo. Pediatr Int, 2001; 43: 493-5.

104. Volpi A, Pica F, Cauletti M, Pana A, Rocchi G. Cytomegalovirus infection in day care centers in rome, italy: Viral excretion in children and occupational risk among workers. J Med Virol, 1988; 26: 119-25.

105. Grosjean J, Trapes L, Hantz S, et al. Human cytomegalovirus quantification in toddlers saliva from day care centers and emergency unit: A feasibility study. J Clin Virol, 2014; 61: 371-7.

106. Huang Y, Guo X, Song Q, et al. Cytomegalovirus shedding in healthy seropositive female college students: A six-month longitudinal study. J Infect Dis, 2018; 217: 1069-1073.

107. Arora N, Novak Z, Fowler KB, Boppana SB, Ross SA. Cytomegalovirus viruria and DNAemia in healthy seropositive women. J Infect Dis, 2010; 202: 1800-3.

108. Picone O, Vauloup-Fellous C, Cordier AG, et al. A series of 238 cytomegalovirus primary infections during pregnancy: Description and outcome. Prenat Diagn, 2013; 33: 751-8.

109. Enders G, Daiminger A, Bader U, Exler S, Enders M. Intrauterine transmission and clinical outcome of 248 pregnancies with primary cytomegalovirus infection in relation to gestational age. J Clin Virol, 2011; 52: 244-6.

110. Schopfer K, Lauber E, Krech U. Congenital cytomegalovirus infection in newborn infants of mothers infected before pregnancy. Arch Dis Child, 1978; 53: 536-9.

111. Simonazzi G, Curti A, Cervi F, et al. Perinatal outcomes of non-primary maternal cytomegalovirus infection: A 15-year experience. Fetal Diagn Ther, 2018; 43: 138-42.

112. Wang C, Zhang X, Bialek S, Cannon MJ. Attribution of congenital cytomegalovirus infection to primary versus non-primary maternal infection. Clin Infect Dis, 2011; 52: e11-3.

113. Papaevangelou V, Christoni Z, Vliora C, et al. Neonatal screening for congenital CMV infection stresses the importance of maternal nonprimary infection even in an area where prenatal serology testing is common. J Matern Fetal Neonatal Med, 2017; 32: 1-4.

114. Griffiths P, Baraniak I, Reeves M. The pathogenesis of human cytomegalovirus. J Pathol, 2015; 235: 288-97.

115. Sinzger C, Digel M, Jahn G. Cytomegalovirus cell tropism. Curr Top Microbiol Immunol, 2008; 325: 63-83.

116. Jackson SE, Mason GM, Wills MR. Human cytomegalovirus immunity and immune evasion. Virus Res, 2011; 157: 151-60.

117. Vanarsdall AL, Johnson DC. Human cytomegalovirus entry into cells. Curr Opin Virol, 2012; 2: 37-42.

118. Sinclair J. Human cytomegalovirus: Latency and reactivation in the myeloid lineage. J Clin Virol, 2008; 41: 180-5.

119. Dupont L, Reeves MB. Cytomegalovirus latency and reactivation: Recent insights into an age old problem. Rev Med Virol, 2016; 26: 75-89.

120. Schleiss MR. Congenital cytomegalovirus infection: Molecular mechanisms mediating viral pathogenesis. Infect Disord Drug Targets, 2011; 11: 449-65.

121. Gabrielli L, Bonasoni MP, Santini D, et al. Congenital cytomegalovirus infection: Patterns of fetal brain damage. Clin Microbiol Infect, 2012; 18: E419-27.

122. Wreghitt TG, Teare EL, Sule O, Devi R, Rice P. Cytomegalovirus infection in immunocompetent patients. Clin Infect Dis, 2003; 37: 1603-6.

123. Nolan N, Halai UA, Regunath H, Smith L, Rojas-Moreno C, Salzer W. Primary cytomegalovirus infection in immunocompetent adults in the United States - A case series. IDCases, 2017; 10: 123-6.

124. Horwitz CA, Henle W, Henle G, et al. Clinical and laboratory evaluation of cytomegalovirus-induced mononucleosis in previously healthy individuals. Report of 82 cases. Medicine (Baltimore), 1986; 65: 124-34.

125. Sridhar S, Chung TWH, Chan JFW, et al. Emergence of cytomegalovirus mononucleosis syndrome among young adults in Hong Kong linked to falling seroprevalence: Results of a 14-year seroepidemiological study. Open Forum Infect Dis, 2018; 5: ofy262.

126. Al-Eyadhy AA, Hasan G, Bassrawi R, et al. Cytomegalovirus associated severe pneumonia, multi-organ failure and ganciclovir associated arrhythmia in immunocompetent child. J Infect Chemother, 2017; 23: 844-7.

127. Doan TT, Phung TT, Pham HV, Pham SH, Nguyen LT. Effect of ganciclovir for the treatment of severe cytomegalovirus-associated pneumonia in children without a specific immunocompromised state. BMC Infect Dis, 2013; 13: 424.

128. Burgener EB, Waggoner J, Pinsky BA, Chen SF. Clinical characteristics and outcomes of pediatric patients with CMV DNA detection in bronchoalveolar lavage fluid. Pediatr Pulmonol, 2017; 52: 112-8.

129. Min CY, Song JY, Jeong SJ. Characteristics and prognosis of hepatic cytomegalovirus infection in children: 10 years of experience at a university hospital in Korea. Korean J Pediatr, 2017; 60: 261-5.

130. Tezer H, Kanik Yuksek S, Gulhan B, Ozkaya Parlakay AN, Tuna Kirsaclioglu C. Cytomegalovirus hepatitis in 49 pediatric patients with normal immunity. Turk J Med Sci, 2016; 46: 1629-33.

131. Jin MJ, Kim Y, Choi EM, et al. Clinical characteristics and treatment courses for cytomegalovirus-associated thrombocytopenia in immunocompetent children after neonatal period. Blood Res, 2018; 53: 110-6.

132. DiMaggio D, Anderson A, Bussel JB. Cytomegalovirus can make immune thrombocytopenic purpura refractory. Br J Haematol, 2009; 146: 104-12.

133. Papagianni A, Economou M, Tsoutsou E, Athanassiou-Metaxa M. CMV-related immune thrombocytopenic purpura or CMV-induced thrombocytopenia? Br J Haematol, 2010; 149: 454-5.

134. Bertoni M, Squizzato A, Foretic M, Zanieri S, Di Natale ME. Cytomegalovirus-associated splanchnic vein thrombosis in immunocompetent patients: A systematic review. Thromb Res, 2018; 168: 104-13.

135. Atzmony L, Halutz O, Avidor B, et al. Incidence of cytomegalovirus-associated thrombosis and its risk factors: A case-control study. Thromb Res, 2010; 126: e439-43.

136. Goodman AL, Murray CD, Watkins J, Griffiths PD, Webster DP. CMV in the gut: A critical review of CMV detection in the immunocompetent host with colitis. Eur J Clin Microbiol Infect Dis, 2015; 34: 13-8.

137. Galiatsatos P, Shrier I, Lamoureux E, Szilagyi A. Meta-analysis of outcome of cytomegalovirus colitis in immunocompetent hosts. Dig Dis Sci, 2005; 50: 609-16.

138. Ljungman P, Boeckh M, Hirsch HH, et al. Definitions of cytomegalovirus infection and disease in transplant patients for use in clinical trials. Clin Infect Dis, 2017; 64: 87-91.

139. Legendre C, Pascual M. Improving outcomes for solid-organ transplant recipients at risk from cytomegalovirus infection: Late-onset disease and indirect consequences. Clin Infect Dis, 2008; 46: 732-40.

140. Vora SB, Englund JA. Cytomegalovirus in immunocompromised children. Curr Opin Infect Dis, 2015; 28: 323-9.

141. Pai SY, Logan BR, Griffith LM, et al. Transplantation outcomes for severe combined immunodeficiency, 2000-2009. N Engl J Med, 2014; 371: 434-46.

142. Natori Y, Alghamdi A, Tazari M, et al. Use of viral load as a surrogate marker in clinical studies of cytomegalovirus in solid organ transplantation: A systematic review and meta-analysis. Clin Infect Dis, 2018; 66: 617-31.

143. Kurath S, Halwachs-Baumann G, Muller W, Resch B. Transmission of cytomegalovirus via breast milk to the prematurely born infant: A systematic review. Clin Microbiol Infect, 2010; 16: 1172-8.

144. Vochem M, Hamprecht K, Jahn G, Speer CP. Transmission of cytomegalovirus to preterm infants through breast milk. Pediatr Infect Dis J, 1998; 17: 53-8.

145. Hamprecht K, Maschmann J, Vochem M, Dietz K, Speer CP, Jahn G. Epidemiology of transmission of cytomegalovirus from mother to preterm infant by breastfeeding. Lancet, 2001; 357: 513-8.

146. Mosca F, Pugni L, Barbi M, Binda S. Transmission of cytomegalovirus. Lancet, 2001; 357: 1800.

147. Yasuda A, Kimura H, Hayakawa M, et al. Evaluation of cytomegalovirus infections transmitted via breast milk in preterm infants with a real-time polymerase chain reaction assay. Pediatrics, 2003; 111: 1333-6.

148. Jim WT, Shu CH, Chiu NC, et al. Transmission of cytomegalovirus from mothers to preterm infants by breast milk. Pediatr Infect Dis J, 2004; 23: 848-51.

149. Omarsdottir S, Casper C, Zweygberg Wirgart B, Grillner L, Vanpee M. Transmission of cytomegalovirus to extremely preterm infants through breast milk. Acta Paediatr, 2007; 96: 492-4.

150. Musonda KG, Nyonda M, Filteau S, Kasonka L, Monze M, Gompels UA. Increased cytomegalovirus secretion and risks of infant infection by breastfeeding duration from maternal human immunodeficiency virus positive compared to negative mothers in sub-Saharan Africa. J Pediatric Infect Dis Soc, 2016; 5: 138-46.

151. Lanzieri TM, Dollard SC, Josephson CD, Schmid DS, Bialek SR. Breast milk-acquired cytomegalovirus infection and disease in VLBW and premature infants. Pediatrics, 2013; 131: e1937-45.

152. Meier J, Lienicke U, Tschirch E, Kruger DH, Wauer RR, Prosch S. Human cytomegalovirus reactivation during lactation and mother-to-child transmission in preterm infants. J Clin Microbiol, 2005; 43: 1318-24.

153. Capretti MG, Lanari M, Lazzarotto T, et al. Very low birth weight infants born to cytomegalovirus-seropositive mothers fed with their mother's milk: A prospective study. J Pediatr, 2009; 154: 842-8.

154. Mussi-Pinhata MM, Yamamoto AY, do Carmo Rego MA, Pinto PC, da Motta MS, Calixto C. Perinatal or early-postnatal cytomegalovirus infection in preterm infants under 34 weeks gestation born to CMV-seropositive

mothers within a high-seroprevalence population. J Pediatr, 2004; 145: 685-8.

155. Miron D, Brosilow S, Felszer K, et al. Incidence and clinical manifestations of breast milk-acquired cytomegalovirus infection in low birth weight infants. J Perinatol, 2005; 25: 299-303.

156. Granstrom M, Leinikki P, Santavuori P, Pettay O. Perinatal cytomegalovirus infection in man. Arch Dis Child, 1977; 52: 354-9.

157. Novakova V, Hamprecht K, Muller AM, Arellano-Galindo J, Ehlen M, Horneff G. Severe postnatal CMV colitis with an extensive colonic stenosis in a 2-month-old male immunocompetent term infant infected via breast milk. J Clin Virol, 2014; 59: 259-63.

158. Paryani SG, Yeager AS, Hosford-Dunn H, et al. Sequelae of acquired cytomegalovirus infection in premature and sick term infants. J Pediatr, 1985; 107: 451-6.

159. Bevot A, Hamprecht K, Krageloh-Mann I, Brosch S, Goelz R, Vollmer B. Long-term outcome in preterm children with human cytomegalovirus infection transmitted via breast milk. Acta Paediatr, 2012; 101: e167-72.

160. Kumar ML, Nankervis GA, Cooper AR, Gold E. Postnatally acquired cytomegalovirus infections in infants of CMV-excreting mothers. J Pediatr, 1984; 104: 669-73.

161. Johnson SJ, Hosford-Dunn H, Paryani S, Yeager A, Malachowski N. Prevalence of sensorineural hearing loss in premature and sick term infants with perinatally acquired cytomegalovirus infection. Ear Hear, 1986; 7: 325-7.

162. Vollmer B, Seibold-Weiger K, Schmitz-Salue C, et al. Postnatally acquired cytomegalovirus infection via breast milk: Effects on hearing and development in preterm infants. Pediatr Infect Dis J, 2004; 23: 322-7.

163. Gunkel J, de Vries LS, Jongmans M, et al. Outcome of preterm infants with postnatal cytomegalovirus infection. Pediatrics, 2018; 141: e20170635.

164. Boppana SB, Pass RF, Britt WJ, Stagno S, Alford CA. Symptomatic congenital cytomegalovirus infection: Neonatal morbidity and mortality. Pediatr Infect Dis J, 1992; 11: 93-9.

165. Kylat RI, Kelly EN, Ford-Jones EL. Clinical findings and adverse outcome in neonates with symptomatic congenital cytomegalovirus (SCCMV) infection. Eur J Pediatr, 2006; 165: 773-8.

166. Vaudry W, Lee BE, Rosychuk RJ. Congenital cytomegalovirus infection in canada: Active surveillance for cases diagnosed by paediatricians. Paediatr Child Health, 2014; 19: e1-5.

167. Noyola DE, Demmler GJ, Nelson CT, et al. Early predictors of neurodevelopmental outcome in symptomatic congenital cytomegalovirus infection. J Pediatr, 2001; 138: 325-31.

168. Conboy TJ, Pass RF, Stagno S, et al. Early clinical manifestations and intellectual outcome in children with symptomatic congenital cytomegalovirus infection. J Pediatr, 1987; 111: 343-8.

169. Mehta V, Balachandran C, Lonikar V. Blueberry muffin baby: A pictoral differential diagnosis. Dermatol Online J, 2008; 14: 8.

170. Boppana SB, Ross SA, Fowler KB. Congenital cytomegalovirus infection: Clinical outcome. Clin Infect Dis, 2013; 57 Suppl 4: S178-81.

171. Hodl S, Aubock L, Reiterer F, Soyer HP, Muller WD. Blueberry muffin baby: The pathogenesis of cutaneous extramedullary hematopoiesis. Hautarzt, 2001; 52: 1035-42.

172. Lautenschlager I, Suni J, Ahonen J, et al. Detection of cytomegalovirus by the early-antigen immunofluorescence test versus conventional tissue culture. Eur J Clin Microbiol Infect Dis, 1989; 8: 610-3.

173. Boppana SB, Ross SA, Shimamura M, et al. Saliva polymerase-chain-reaction assay for cytomegalovirus screening in newborns. N Engl J Med, 2011; 364: 2111-8.

174. Yamamoto AY, Mussi-Pinhata MM, Marin LJ, Brito RM, Oliveira PF, Coelho TB. Is saliva as reliable as urine for detection of cytomegalovirus DNA for neonatal screening of congenital CMV infection? J Clin Virol, 2006; 36: 228-30.

175. Koontz D, Baecher K, Amin M, Nikolova S, Gallagher M, Dollard S. Evaluation of DNA extraction methods for the detection of cytomegalovirus in dried blood spots. J Clin Virol, 2015; 66: 95-9.

176. Boppana SB, Ross SA, Novak Z, et al. Dried blood spot real-time polymerase chain reaction assays to screen newborns for congenital cytomegalovirus infection. JAMA, 2010; 303: 1375-82.

177. Barbi M, Binda S, Primache V, et al. Cytomegalovirus DNA detection in guthrie cards: A powerful tool for diagnosing congenital infection. J Clin Virol, 2000; 17: 159-65.

178. de Vries JJ, Claas EC, Kroes AC, Vossen AC. Evaluation of DNA extraction methods for dried blood spots in the diagnosis of congenital cytomegalovirus infection. J Clin Virol, 2009; 46 Suppl 4: S37-42.

179. Bilavsky E, Watad S, Levy I, et al. Positive IgM in congenital CMV infection. Clin Pediatr (Phila), 2017; 56: 371-5.

180. Revello MG, Zavattoni M, Baldanti F, Sarasini A, Paolucci S, Gerna G. Diagnostic and prognostic value of human cytomegalovirus load and IgM antibody in blood of congenitally infected newborns. J Clin Virol, 1999; 14: 57-66.

181. Zavattoni M, Lombardi G, Rognoni V, et al. Maternal, fetal, and neonatal parameters for prognosis and counseling of HCMV congenital infection. J Med Virol, 2014; 86: 2163-70.

182. Ancora G, Lanari M, Lazzarotto T, et al. Cranial ultrasound scanning and prediction of outcome in newborns with congenital cytomegalovirus infection. J Pediatr, 2007; 150: 157-61.

183. Capretti MG, Lanari M, Tani G, et al. Role of cerebral ultrasound and magnetic resonance imaging in newborns with congenital cytomegalovirus infection. Brain Dev, 2014; 36: 203-11.

184. Giannattasio A, Bruzzese D, Di Costanzo P, et al. Neuroimaging profiles and neurodevelopmental outcome in infants with congenital cytomegalovirus infection. Pediatr Infect Dis J, 2018; 37: 1028-33.

185. Lanari M, Capretti MG, Lazzarotto T, et al. Neuroimaging in CMV congenital infected neonates: How and when. Early Hum Dev, 2012; 88 Suppl 2: S3-5.

186. Fowler KB, Dahle AJ, Boppana SB, Pass RF. Newborn hearing screening: Will children with hearing loss caused by congenital cytomegalovirus infection be missed? J Pediatr, 1999; 135: 60-4.

187. Boppana SB, Fowler KB, Pass RF, et al. Congenital cytomegalovirus infection: Association between virus burden in infancy and hearing loss. J Pediatr, 2005; 146: 817-23.

188. Ross SA, Fowler KB, Ashrith G, et al. Hearing loss in children with congenital cytomegalovirus infection born to mothers with preexisting immunity. J Pediatr, 2006; 148: 332-6.

189. Harris S, Ahlfors K, Ivarsson S, Lernmark B, Svanberg L. Congenital cytomegalovirus infection and sensorineural hearing loss. Ear Hear, 1984; 5: 352-5.

190. Teissier N, Delezoide AL, Mas AE, et al. Inner ear lesions in congenital cytomegalovirus infection of human fetuses. Acta Neuropathol, 2011; 122: 763-74.

191. Gabrielli L, Bonasoni MP, Santini D, et al. Human fetal inner ear involvement in congenital cytomegalovirus infection. Acta Neuropathol Commun, 2013; 1: 63.

192. Goderis J, De Leenheer E, Smets K, Van Hoecke H, Keymeulen A, Dhooge I. Hearing loss and congenital CMV infection: A systematic review. Pediatrics, 2014; 134: 972-82.

193. Foulon I, Naessens A, Faron G, Foulon W, Jansen AC, Gordts F. Hearing thresholds in children with a congenital CMV infection: A prospective study. Int J Pediatr Otorhinolaryngol, 2012; 76: 712-7.

194. De Kegel A, Maes L, Dhooge I, van Hoecke H, De Leenheer E, Van Waelvelde H. Early motor development of children with a congenital cytomegalovirus infection. Res Dev Disabil, 2016; 48: 253-61.

195. Goderis J, Keymeulen A, Smets K, et al. Hearing in children with congenital cytomegalovirus infection: Results of a longitudinal study. J Pediatr, 2016; 172: 110-115.e2.

196. Williamson WD, Demmler GJ, Percy AK, Catlin FI. Progressive hearing loss in infants with asymptomatic congenital cytomegalovirus infection. Pediatrics, 1992; 90: 862-6.

197. Dreher AM, Arora N, Fowler KB, et al. Spectrum of disease and outcome in children with symptomatic congenital cytomegalovirus infection. J Pediatr, 2014; 164: 855-9.

198. Ross SA, Fowler KB, Ashrith G, et al. Hearing loss in children with congenital cytomegalovirus infection born to mothers with preexisting immunity. J Pediatr, 2006; 148: 332-6.

199. Ahlfors K, Ivarsson SA, Harris S, et al. Congenital cytomegalovirus infection and disease in sweden and the relative importance of primary and secondary maternal infections. Preliminary findings from a prospective study. Scand J Infect Dis, 1984; 16: 129-37.

200. Townsend CL, Forsgren M, Ahlfors K, Ivarsson SA, Tookey PA, Peckham CS. Long-term outcomes of congenital cytomegalovirus infection in Sweden and the United Kingdom. Clin Infect Dis, 2013; 56: 1232-9.

201. Bartlett AW, McMullan B, Rawlinson WD, Palasanthiran P. Hearing and neurodevelopmental outcomes for children with asymptomatic congenital cytomegalovirus infection: A systematic review. Rev Med Virol, 2017. Doi:10.1002/rmv.1938. Epub ahead of print.

202. Kumar ML, Nankervis GA, Jacobs IB, et al. Congenital and postnatally acquired cytomegalovirus infections: Long-term follow-up. J Pediatr, 1984; 104: 674-9.

203. Conboy TJ, Pass RF, Stagno S, et al. Intellectual development in school-aged children with asymptomatic congenital cytomegalovirus infection. Pediatrics, 1986; 77: 801-6.

204. Pearl KN, Preece PM, Ades A, Peckham CS. Neurodevelopmental assessment after congenital cytomegalovirus infection. Arch Dis Child, 1986; 61: 323-6.

205. Kashden J, Frison S, Fowler K, Pass RF, Boll TJ. Intellectual assessment of children with asymptomatic congenital cytomegalovirus infection. J Dev Behav Pediatr, 1998; 19: 254-9.

206. Lopez AS, Lanzieri TM, Claussen AH, et al. Intelligence and academic achievement with asymptomatic congenital cytomegalovirus infection. Pediatrics, 2017; 140: 5: e20171517.

207. Ivarsson SA, Lernmark B, Svanberg L. Ten-year clinical, developmental, and intellectual follow-up of children with congenital cytomegalovirus infection without neurologic symptoms at one year of age. Pediatrics, 1997; 99: 800-3.

208. Capretti MG, Marsico C, Guidelli Guidi S, et al. Neonatal and long-term ophthalmological findings in infants with symptomatic and asymptomatic congenital cytomegalovirus infection. J Clin Virol, 2017; 97: 59-63.

209. Jin HD, Demmler-Harrison GJ, Coats DK, et al. Long-term visual and ocular sequelae in patients with congenital cytomegalovirus infection. Pediatr Infect Dis J, 2017; 36: 877-82.

210. Lanzieri TM, Leung J, Caviness AC, et al. Long-term outcomes of children with symptomatic congenital cytomegalovirus disease. J Perinatol, 2017; 37: 875-80.

211. Tear Fahnehjelm K, Olsson M, Fahnehjelm C, Lewensohn-Fuchs I, Karltorp E. Chorioretinal scars and visual deprivation are common in children with cochlear implants after congenital cytomegalovirus infection. Acta Paediatr, 2015; 104: 693-700.

212. Coats DK, Demmler GJ, Paysse EA, Du LT, Libby C. Ophthalmologic findings in children with congenital cytomegalovirus infection. J AAPOS, 2000; 4: 110-6.

213. Karltorp E, Lofkvist U, Lewensohn-Fuchs I, et al. Impaired balance and neurodevelopmental disabilities among children with congenital cytomegalovirus infection. Acta Paediatr, 2014; 103: 1165-73.

214. Stagno S, Pass RF, Thomas JP, Navia JM, Dworsky ME. Defects of tooth structure in congenital cytomegalovirus infection. Pediatrics, 1982; 69: 646-8.

215. Stagno S, Pass RF, Dworsky ME, et al. Congenital cytomegalovirus infection: The relative importance of primary and recurrent maternal infection. N Engl J Med, 1982; 306: 945-9.

216. Yamamoto AY, Mussi-Pinhata MM, Isaac Mde L, et al. Congenital cytomegalovirus infection as a cause of sensorineural hearing loss in a highly immune population. Pediatr Infect Dis J, 2011; 30: 1043-6.

217. Boppana SB, Fowler KB, Britt WJ, Stagno S, Pass RF. Symptomatic congenital cytomegalovirus infection in infants born to mothers with preexisting immunity to cytomegalovirus. Pediatrics, 1999; 104: 55-60.

218. Ahlfors K, Ivarsson SA, Harris S. Secondary maternal cytomegalovirus infection—A significant cause of congenital disease. Pediatrics, 2001; 107: 1227-8.

219. Britt WJ. Maternal immunity and the natural history of congenital human cytomegalovirus infection. Viruses, 2018; 10(8):405

220. Giannattasio A, Di Costanzo P, De Matteis A, et al. Outcomes of congenital cytomegalovirus disease following maternal primary and non-primary infection. J Clin Virol, 2017; 96: 32-6.

221. Ross SA, Novak Z, Fowler KB, Arora N, Britt WJ, Boppana SB. Cytomegalovirus blood viral load and hearing loss in young children with congenital infection. Pediatr Infect Dis J, 2009; 28: 588-92.

222. Lanari M, Lazzarotto T, Venturi V, et al. Neonatal cytomegalovirus blood load and risk of sequelae in symptomatic and asymptomatic congenitally infected newborns. Pediatrics, 2006; 117: e76-83.

223. Forner G, Abate D, Mengoli C, Palu G, Gussetti N. High cytomegalovirus (CMV) DNAemia predicts CMV sequelae in asymptomatic congenitally infected newborns born to women with primary infection during pregnancy. J Infect Dis, 2015; 212: 67-71.

224. Noyola DE, Demmler GJ, Williamson WD, et al. Cytomegalovirus urinary excretion and long-term outcome in children with congenital cytomegalovirus infection. Congenital CMV Longitudinal Study Group. Pediatr Infect Dis J, 2000; 19: 505-10.

225. Rosenthal LS, Fowler KB, Boppana SB, et al. Cytomegalovirus shedding and delayed sensorineural hearing loss: Results from longitudinal follow-up of children with congenital infection. Pediatr Infect Dis J, 2009; 28: 515-20.

226. Whitley RJ, Cloud G, Gruber W, et al. Ganciclovir treatment of symptomatic congenital cytomegalovirus infection: Results of a phase II study. National institute of allergy and infectious diseases collaborative antiviral study group. J Infect Dis, 1997; 175: 1080-6.

227. Kimberlin DW, Lin CY, Sanchez PJ, et al. Effect of ganciclovir therapy on hearing in symptomatic congenital cytomegalovirus disease involving the central nervous system: A randomized, controlled trial. J Pediatr, 2003; 143: 16-25.

228. Oliver SE, Cloud GA, Sanchez PJ, et al. Neurodevelopmental outcomes following ganciclovir therapy in symptomatic congenital cytomegalovirus infections involving the central nervous system. J Clin Virol, 2009; 46 Suppl 4: S22-6.

229. Amir J, Wolf DG, Levy I. Treatment of symptomatic congenital cytomegalovirus infection with intravenous ganciclovir followed by long-term oral valganciclovir. Eur J Pediatr, 2010; 169: 1061-7.

230. Kimberlin DW, Jester PM, Sanchez PJ, et al. Valganciclovir for symptomatic congenital cytomegalovirus disease. N Engl J Med, 2015; 372: 933-43.

231. Meyer K, Anderson J, Venturelli A, et al. Identification of a critical window for Ganciclovir-induced disruption of Testicular development in Rats. Toxicol Sci, 2018; 162:488-98.

232. Neyts J, Jahne G, Andrei G, Snoeck R, Winkler I, De Clercq E. In vivo antiherpesvirus activity of N-7-substituted acyclic nucleoside analog 2-amino-7-[(1,3-dihydroxy-2-propoxy)methyl]purine. Antimicrob Agents Chemother, 1995; 39: 56-60.

233. Faqi AS, Klug A, Merker HJ, Chahoud I. Ganciclovir induces reproductive hazards in male rats after short-term exposure. Hum Exp Toxicol, 1997; 16: 505-11.

234. Nihi F, Moreira D, Santos Lourenco AC, et al. Testicular effects following in utero exposure to the antivirals acyclovir and ganciclovir in rats. Toxicol Sci, 2014; 139: 220-33.

235. Rawlinson WD, Boppana SB, Fowler KB, et al. Congenital cytomegalovirus infection in pregnancy and the neonate: Consensus recommendations for prevention, diagnosis, and therapy. Lancet Infect Dis, 2017; 17: e177-88.

236. Luck SE, Wieringa JW, Blazquez-Gamero D, et al. Congenital cytomegalovirus: A European expert consensus statement on diagnosis and management. Pediatr Infect Dis J, 2017; 36: 1205-13.

237. Adler SP, Finney JW, Manganello AM, Best AM. Prevention of child-to-mother transmission of cytomegalovirus among pregnant women. J Pediatr, 2004; 145: 485-91.

238. Vauloup-Fellous C, Picone O, Cordier AG, et al. Does hygiene counseling have an impact on the rate of CMV primary infection during pregnancy? Results of a 3-year prospective study in a French hospital. J Clin Virol, 2009; 46 Suppl 4: S49-53.

239. Revello MG, Tibaldi C, Masuelli G, et al. Prevention of primary cytomegalovirus infection in pregnancy. EBioMedicine, 2015; 2: 1205-10.

240. Nigro G, Adler SP, La Torre R, Best AM, Congenital Cytomegalovirus Collaborating Group. Passive immunization during pregnancy for congenital cytomegalovirus infection. N Engl J Med, 2005; 353: 1350-62.

241. Nigro G, Adler SP, Parruti G, et al. Immunoglobulin therapy of fetal cytomegalovirus infection occurring in the first half of pregnancy—a case-control study of the outcome in children. J Infect Dis, 2012; 205: 215-27.

242. Buxmann H, Stackelberg OM, Schlosser RL, et al. Use of cytomegalovirus hyperimmunoglobulin for prevention of congenital cytomegalovirus disease: A retrospective analysis. J Perinat Med, 2012; 40: 439-46.

243. Visentin S, Manara R, Milanese L, et al. Early primary cytomegalovirus infection in pregnancy: Maternal hyperimmunoglobulin therapy improves outcomes among infants at 1 year of age. Clin Infect Dis, 2012; 55: 497-503.

244. Revello MG, Lazzarotto T, Guerra B, et al. A randomized trial of hyperimmune globulin to prevent congenital cytomegalovirus. N Engl J Med, 2014; 370: 1316-26.

245. Korndewal MJ, Oudesluys-Murphy AM, Kroes ACM, van der Sande MAB, de Melker HE, Vossen ACTM. Long-term impairment attributable to congenital cytomegalovirus infection: A retrospective cohort study. Dev Med Child Neurol, 2017; 59: 1261-8.

246. Working Group set up by the Finnish Medical Society Duodecim and the Finnish Dental Society Apollonia. Caries (control). Current care guideline. Finnish Medical Society Duodecim, 2014.

247. Fowler KB, McCollister FP, Sabo DL, et al. A targeted approach for congenital cytomegalovirus screening within newborn hearing screening. Pediatrics, 2017; 139: pii: e20162128.

248. Cannon MJ, Griffiths PD, Aston V, Rawlinson WD. Universal newborn screening for congenital CMV infection: What is the evidence of potential benefit? Rev Med Virol, 2014; 24: 291-307.

249. Demmler-Harrison GJ. Congenital cytomegalovirus infection: The elephant in our living room. JAMA Pediatr, 2016; 170: 1142-4.

250. Kennedy CR, McCann DC, Campbell MJ, et al. Language ability after early detection of permanent childhood hearing impairment. N Engl J Med, 2006; 354: 2131-41.

251. Gantt S, Dionne F, Kozak FK, et al. Cost-effectiveness of universal and targeted newborn screening for congenital cytomegalovirus infection. JAMA Pediatr, 2016; 170: 1173-80.

252. Bergevin A, Zick CD, McVicar SB, Park AH. Cost-benefit analysis of targeted hearing directed early testing for congenital cytomegalovirus infection. Int J Pediatr Otorhinolaryngol, 2015; 79: 2090-3.

253. Lagrou K, Bodeus M, Van Ranst M, Goubau P. Evaluation of the new architect cytomegalovirus immunoglobulin M (IgM), IgG, and IgG avidity assays. J Clin Microbiol, 2009; 47: 1695-9.

254. Juhl D, Vockel A, Luhm J, Ziemann M, Hennig H, Gorg S. Comparison of the two fully automated anti-HCMV IgG assays: Abbott architect CMV IgG assay and biotest anti-HCMV recombinant IgG ELISA. Transfus Med, 2013; 23: 187-94.

255. Gorzer I, Kerschner H, Jaksch P, et al. Virus load dynamics of individual CMV-genotypes in lung transplant recipients with mixed-genotype infections. J Med Virol, 2008; 80: 1405-14.

256. Pang X, Humar A, Preiksaitis JK. Concurrent genotyping and quantitation of cytomegalovirus gB genotypes in solid-organ-transplant recipients by use of a real-time PCR assay. J Clin Microbiol, 2008; 46: 4004-10.

257. Novak Z, Ross SA, Patro RK, et al. Cytomegalovirus strain diversity in seropositive women. J Clin Microbiol, 2008; 46: 882-6.

258. Griffiths R, Huntley M. The Griffiths mental development scales from birth to 2 years. Mannual. 1996 Revision. Oxford, UK: The Test Agency Limited, 1996.

259. Sankilampi U, Hannila ML, Saari A, Gissler M, Dunkel L. New population-based references for birth weight, length, and head circumference in singletons and twins from 23 to 43 gestation weeks. Ann Med, 2013; 45: 446-54.

260. Statistics Finland's PX-Web databases. Available at: https://pxnet2.stat.fi/PXWeb/pxweb/fi/StatFin/

261. Chen SF, Holmes TH, Slifer T, et al. Longitudinal kinetics of cytomegalovirus-specific T-cell immunity and viral replication in infants with congenital cytomegalovirus infection. J Pediatric Infect Dis Soc, 2016; 5: 14-20.

262. Enders G, Daiminger A, Lindemann L, et al. Cytomegalovirus (CMV) seroprevalence in pregnant women, bone marrow donors and adolescents in germany, 1996-2010. Med Microbiol Immunol, 2012; 201: 303-9.

263. Taniguchi K, Watanabe N, Sato A, et al. Changes in cytomegalovirus seroprevalence in pregnant Japanese women—A 10-year single center study. J Clin Virol, 2014; 59: 192-4.

264. Stagno S, Cloud GA. Working parents: The impact of day care and breast-feeding on cytomegalovirus infections in offspring. Proc Natl Acad Sci U S A, 1994; 91: 2384-9.

265. Kansallinen imetyksen edistämisen asiantuntijaryhmä. Imetyksen edistäminen Suomessa. Toimintaohjelma 2009-2012. Yliopistopaino, Helsinki, 2009.

266. Uusitalo L, Nyberg H, Pelkonen M, Sarlio-Lähteenkorva S, Hakulinen-Viitanen T, Virtanen S. Imeväisikäisten ruokinta Suomessa vuonna 2010. Juvenes Print, Helsinki 2012.

267. Romero-Gomez MP, Cabrera M, Montes-Bueno MT, et al. Evaluation of cytomegalovirus infection in low-birth weight children by breast milk using a real-time polymerase chain reaction assay. J Med Virol, 2015; 87: 845-50.

268. Säkkinen S, Kuoppala T. Tilastoraportti varhaiskasvatus 2017. THL, 2018.

269. Santos DV, Souza MM, Goncalves SH, et al. Congenital cytomegalovirus infection in a neonatal intensive care unit in Brazil evaluated by PCR and association with perinatal aspects. Rev Inst Med Trop Sao Paulo, 2000; 42: 129-32.

270. Panhani S, Heinonen KM. Screening for congenital cytomegalovirus infection among preterm infants born before the 34th gestational week in Finland. Scand J Infect Dis, 1994; 26: 375-8.

271. Stagno S, Reynolds DW, Amos CS, et al. Auditory and visual defects resulting from symptomatic and subclinical congenital cytomegaloviral and toxoplasma infections. Pediatrics, 1977; 59: 669-78.

272. Dahle AJ, Fowler KB, Wright JD, Boppana SB, Britt WJ, Pass RF. Longitudinal investigation of hearing disorders in children with congenital cytomegalovirus. J Am Acad Audiol, 2000; 11: 283-90.

273. Ahlfors K, Forsgren M, Ivarsson SA, Harris S, Svanberg L. Congenital cytomegalovirus infection: On the relation between type and time of maternal infection and infant's symptoms. Scand J Infect Dis, 1983; 15: 129-38.

274. Casteels A, Naessens A, Gordts F, De Catte L, Bougatef A, Foulon W. Neonatal screening for congenital cytomegalovirus infections. J Perinat Med, 1999; 27: 116-21.

275. Faure-Bardon V, Magny JF, Parodi M, et al. Sequelae of congenital cytomegalovirus (cCMV) following maternal primary infection are limited to those acquired in the first trimester of pregnancy. Clin Infect Dis, 2018 Dec 31. Epub ahead of print.

276. Vadini F, Tracanna E, Polilli E, et al. Post-traumatic stress in pregnant women with primary cytomegalovirus infection and risk of congenital infection in newborns. BJPsych Open, 2016; 2: 373-6.

277. Mujtaba G, Khurshid A, Sharif S, et al. Distribution of cytomegalovirus genotypes among neonates born to infected mothers in Islamabad, Pakistan. PLoS One, 2016; 11: e0156049.

278. Paradowska E, Studzinska M, Suski P, et al. Human cytomegalovirus UL55, UL144, and US28 genotype distribution in infants infected congenitally or postnatally. J Med Virol, 2015; 87: 1737-48.

279. de Vries JJ, Wessels E, Korver AM, et al. Rapid genotyping of cytomegalovirus in dried blood spots by multiplex real-time PCR assays targeting the envelope glycoprotein gB and gH genes. J Clin Microbiol, 2012; 50: 232-7.

280. Trincado DE, Scott GM, White PA, Hunt C, Rasmussen L, Rawlinson WD. Human cytomegalovirus strains associated with congenital and perinatal infections. J Med Virol, 2000; 61: 481-7.

281. Barbi M, Binda S, Caroppo S, et al. CMV gB genotypes and outcome of vertical transmission: Study on dried blood spots of congenitally infected babies. J Clin Virol, 2001; 21: 75-9.

282. Picone O, Costa JM, Leruez-Ville M, Ernault P, Olivi M, Ville Y. Cytomegalovirus (CMV) glycoprotein B genotype and CMV DNA load in the amniotic fluid of infected fetuses. Prenat Diagn, 2004; 24: 1001-6.

283. Branas P, Blazquez-Gamero D, Galindo A, et al. Cytomegalovirus genotype distribution among congenitally and postnatally infected patients: Association of particular glycoprotein (g)B and gN types with symptomatic disease. Open Forum Infect Dis, 2015; 2: ofv151.

284. Bale JF, Jr, Murph JR, Demmler GJ, Dawson J, Miller JE, Petheram SJ. Intrauterine cytomegalovirus infection and glycoprotein B genotypes. J Infect Dis, 2000; 182: 933-6.

285. Tortorella D, Gewurz BE, Furman MH, Schust DJ, Ploegh HL. Viral subversion of the immune system. Annu Rev Immunol, 2000; 18: 861-926.

286. Gonzalez-Sanchez HM, Alvarado-Hernandez DL, Guerra-Palomares S, Garcia-Sepulveda CA, Noyola DE. Cytomegalovirus glycoprotein B genotypes in Mexican children and women. Intervirology, 2015; 58: 115-21.

287. Arista S, De Grazia S, Giammanco GM, Di Carlo P, Iannitto E. Human cytomegalovirus glycoprotein B genotypes in immunocompetent, immunocompromised, and congenitally infected Italian populations. Arch Virol, 2003; 148: 547-54.

288. Arellano-Galindo J, Villanueva-Garcia D, Cruz-Ramirez JL, et al. Detection and gB genotyping of CMV in Mexican preterm infants in the context of maternal seropositivity. J Infect Dev Ctries, 2014; 8: 758-67.

289. Gorzer I, Trajanoski S, Popow-Kraupp T, Puchhammer-Stockl E. Analysis of human cytomegalovirus strain populations in urine samples of newborns by ultra deep sequencing. J Clin Virol, 2015; 73: 101-4.

290. Pass RF, Stagno S, Britt WJ, Alford CA. Specific cell-mediated immunity and the natural history of congenital infection with cytomegalovirus. J Infect Dis, 1983; 148: 953-61.

291. Stowell JD, Forlin-Passoni D, Radford K, et al. Cytomegalovirus survival and transferability and the effectiveness of common hand-washing agents against cytomegalovirus on live human hands. Appl Environ Microbiol, 2014; 80: 455-61.

292. Murph JR, Bale JF, Jr, Murray JC, Stinski MF, Perlman S. Cytomegalovirus transmission in a midwest day care center: Possible relationship to child care practices. J Pediatr, 1986; 109: 35-9.

293. Datta S, Budhauliya R, Das B, Chatterjee S, Vanlalhmuaka, Veer V. Next-generation sequencing in clinical virology: Discovery of new viruses. World J Virol, 2015; 4: 265-76.